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Archives of Internal Medicine

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EPITHELIOMATA OF THYMIC ORIGIN*

DOUGLAS SYMMERS, M.D.
AND
B. M. VANCE, M.D.
NEW YORK

The histogenesis of the thymus is still a subject of debate. Most investigators are agreed that the reticulum and Hassall's corpuscles are epithelial in origin. The derivation of the chief elements of the organ, however, namely, the small cells, has not been determined. For example, Maximow [1] believes that early in the process of development the thymus becomes invaded by mesenchymal elements which differentiate into lymphocytes and that these accumulate in such numbers as to give to the organ the appearance of a lymphoid structure. Maximow's belief in the lymphocytic nature of the small cells is shared by Hammar [2] and Schaffer,[3] but is opposed by Stöhr [4] and Pappenheimer,[5] who regard them as epithelial. It seems to us that the conception of the small thymic cell as a lymphocyte is more in harmony with common knowledge of the pathology of the gland, especially its tumors and, if Maximow's conception is correct, it follows that the parenchyma of the thymus possesses a dual embryogenesis—the lymphoid cells being derived from the mesoderm and the epithelium from the ectoderm, a distinction which the thymus shares with the suprarenal capsule, whose cortical cells are mesodermal, while those of the medulla are ectodermal. Appreciation of the dual origin of the parenchyma of these organs is useful in approaching the study of the tumors that arise from them. For example, in the suprarenal, two clinically and otherwise separable varieties of malignant tumor may be distinguished—one, the so-called suprarenal hypernephroma; the second takes its origin from residual neuroblasts in the medulla, the so-called neuroblastoma, and is consequently ectodermal. Likewise, it is possible broadly to divide the tumors of the thymic parenchyma into two groups—the members of one group springing from the preponderating cell of the adult thymus, namely, the lymphocyte; the members of the other group arising from

* From the Pathological Laboratories, Bellevue Hospital.

1. Maximow: Arch. f. mikr. Anat. **24**:525, 1909.
2. Hammar: Arch. f Anat. u. Physiol. (Anat. Abth.), 1907.
3. Schaffer: Sitzbericht. kaiserl. Akad. Wissenach., Wien. **118**: 1909.
4. Stöhr: Anat. Hefte **41**: 1910.
5. Pappenheimer: J. M. Research **17**:1, 1910.

that cell which originally dominated the thymus but which, as development proceeds, is projected into the background, that is to say, the epithelial cell.

The thymic lymphosarcomas are divisible into two histologic groups. The first and prevailing type of tumor (a) is made up of lymphoid cells which are arranged diffusely or are gathered in small packets separated from one another by delicate strands of connective tissue. Most of the pure lymphocytic tumors are sparsely vascularized, but in occasional instances blood vessels are present in vast numbers and, in these circumstances, the mass may pulsate, simulating aneurysm. In other cases the lymphocytic growth may persist for a variable length of time, with or without pressure symptoms referable to the upper part of the thorax, and suddenly commence to pour lymphocytes into the circulation in such numbers as to constitute a form of acute leukemia, and thus to terminate life within a few weeks.

(b) The second variety of thymic lymphosarcoma is histologically comparable to Hodgkin's disease and is composed of lymphoid cells among which are giant cells of the myeloid type, together with eosinophils and eosinophilic myelocytes.[6]

To the lymphoid tumors it has been proposed to apply the designation thymoma, but this appellation, it seems to us, is permissible, if at all, only when it is used to denote a tumor that more or less faithfully reproduces the structure of the thymus as a whole, including lymphocytes, epithelial reticulum cells and Hassall's bodies. As far as we have been able to gather, such a tumor has not thus far been differentiated and until it is and until the histogenesis of the thymus is settled, the designation of thymoma must be regarded as misleading and as tending to complicate rather than to clarify the already hopelessly involved nomenclature of the neoplasms. To justify the term thymoma it is not sufficient merely to demonstrate, for example, the presence of Hassall's bodies in the primary growth, but to show that these bodies are newly formed and not residua of the originally normal thymus, and that they occur in association with other structural counterfeits of the thymic tissues. In this connection it is worth while to note that in our search of the literature and in our personal experience with the study of thymus tumors, we have failed to find a case where Hassall's bodies occurred in any of the secondary formations. The growing practice of naming tumors according to the organs from which they spring is a procedure of doubtful value. If the nomenclature of tumors is to be satisfactorily recast, we must seek simpler criteria. For example, hypernephroma means nothing other than a tumor arising somewhere above the kidney; hepatoma conveys no intelligible con-

6. Symmers: New York M. J. **18**:971, 1911.

ception of the cellular genesis of a tumor of the liver; and thymoma does not serve in the least to simplify our knowledge of the more remote origin of the tumors of the thymus. We might more profitably busy ourselves with the task of correlating clinical events with the growth and distribution of tumors and with their cellular derivation as opposed to the organs in which they arise.

In the developing thymus the glandular structures are separated and compressed by infiltrating lymphocytes to such an extent that the

Fig. 1.—Low power photomicrograph showing the general architecture of the thymic growth. Above are small cells arranged in alveoli. Below are masses of flattened cells in one of which is a group of small cells.

epithelium is reduced to a combination of delicate reticulum and circumscribed collections of cells known as Hassall's bodies, the latter bearing a resemblance to the epithelial pearls that occur in certain inflammatory lesions of the skin and in many of the epitheliomas of the skin and mucocutaneous junctions. From the observations of Paviot and Gerest, Thiroloix and Debre, and Rubaschow, as well as

from the study here recorded, it seems to be established that there is a variety of tumor of the thymus gland that arises from the epithelial remnants in question, and that the growth shows a tendency to reproduce Hassall's bodies—in other words, that there is a form of epithelioma of thymic origin. Rubaschow divides thymus tumors of this description into two groups—one composed of large flat cells of the epidermal type, the other made up of smaller, rounded epithelial cells, the tumor showing no disposition to develop concentric formations

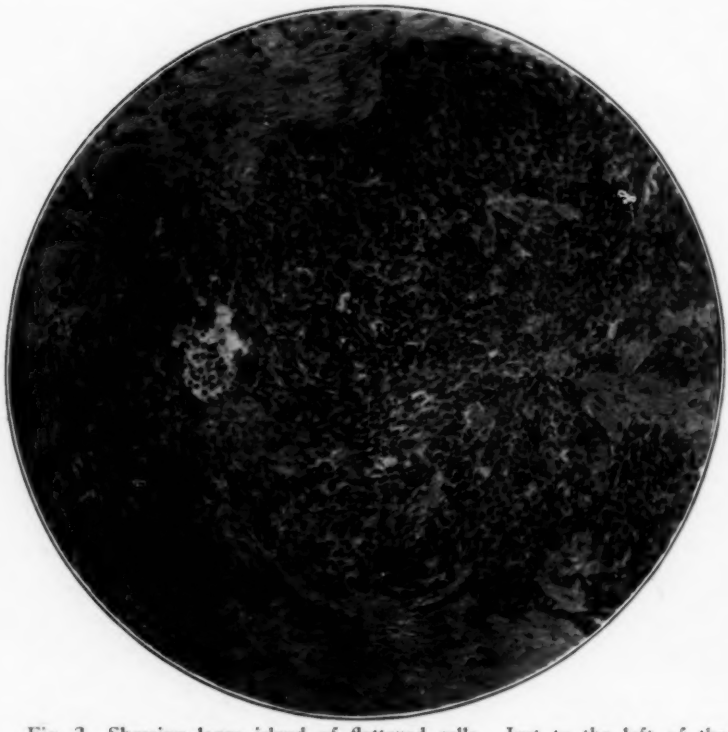

Fig. 2.—Showing large island of flattened cells. Just to the left of the center is a small whorllike formation.

comparable to Hassall's bodies. In the neoplasm that we describe herein, the histology is rather more complex and partakes of the nature of both the large and small cell types of Rubaschow.

Only three examples of primary thymic epithelioma are to be found in the literature. A fourth is recorded in this paper. Apparently, the first genuine primary epithelioma of the thymus to be described was that of Paviot and Gerest.[7] The patient was a domestic, aged 52 years.

7. Paviot & Gerest: Arch. méd. expér. **8**:699, 1896.

The patient stated that three months before admission to the hospital she began to suffer from retrosternal pains which gradually grew worse and became associated with signs of suffocation and edema of the legs. At the time of admission, the patient was intensely dyspneic and cyanotic, and complained of pains behind the sternum and of cough and expectoration. The voice was of metallic quality. Emaciation was pronounced. At the necropsy, a massive tumor was found

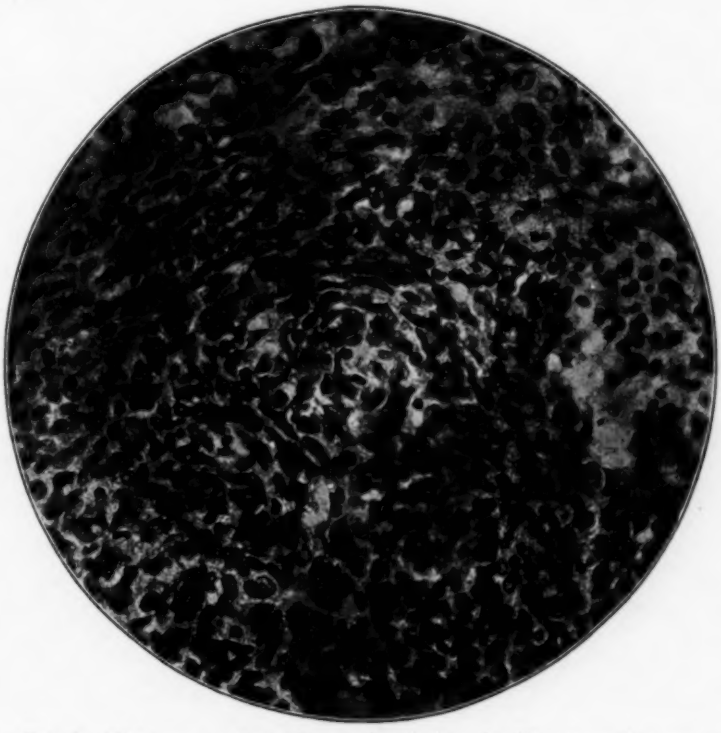

Fig. 3.—High power photomicrograph of the whorllike formation represented in Figure 2. Note the shape and arrangement of the cells at the center of the whorl and the general resemblance to Hassall's bodies.

above the heart in the region of the thymus. The growth measured 16x11x13 cm. and was pyramidal in shape, firm in consistence, and the left lung was attached to it. Microscopic examination revealed a neoplasm the cells of which were of different sizes and arranged in unequal strands and columns. The cells were large and some of them were hyperchromatic and of the giant variety. In places the growth showed a tendency to form concentric bodies resembling Hassall's cor-

puscles. The stroma of the tumor was abundant and poorly vascularized. One of the kidneys contained a minute metastatic nodule which, on microscopic examination, was found to possess much the same morphology as that of the parent growth.

The thymic epithelioma described by Thiroloix and Debre [8] occurred in a man, 56 years of age. The patient complained of retrosternal pains, dyspnea and loss of weight and strength. The total duration of illness was two years. At necropsy, a tumor was found occupying the

Fig. 4.—Field showing extreme variations in the arrangement and morphology of the tumor cells and the tendency to form whorls resembling Hassall's bodies.

thymic area. The growth was roughly triangular in shape with the base upward and was described as about the size of an adult head. It was whitish in color and of cartilaginoid consistence, infiltrated the upper borders of the lungs and sternum, and was adherent to the trachea, the pericardium and auricles of the heart, and to the large

8. Thiroloix & Debre: Arch. méd. expér. **19**:668, 1907.

vessels and nerves of the vicinity. No metastases were found. Microscopic examination showed that the tumor cells were from 5 to 30 micromillimeters in size, polyhedral in outline, many of them hyperchromatic. They were arranged in solid cords, showing in places attempts to form concentric bodies with central areas of keratinization. The illustration which accompanies their paper indicates plainly that the tumor belongs in the group of the epitheliomas.

Fig. 5.—High power photomicrograph of the hyperchromatic cells shown to the right of the middle in Figure 4. Note the extraordinary variations in the morphology of the cells.

In the case of thymic epithelioma recorded by Rubaschow,[9] the patient was a man, 62 years of age. During life he presented no signs referable to a growth in the thymic region. At necropsy, a mass was found between the sternum and the pericardium, corresponding to the normal location of the thymus. It measured 12x7x5 cm. and was of firm consistence, whitish in color, and adherent to the pericardium,

9. Rubaschow: Virchows Arch. f. path. Anat. **206**:141, 1911.

the hilus of both lungs, and the capsule of the thyroid gland. Microscopically, the tumor was found to consist of cells collected into solid groups lying in a supporting network of connective tissue. The cells were rounded or somewhat irregular in shape and noticeably larger than the lymphocyte. Lying among them were remnants of thymic tissue in the form of Hassall's corpuscles.

In the case that we studied at Bellevue Hospital, the patient was a man, 58 years of age, a painter by occupation, who was admitted to

Fig. 6.—Showing the presence of metastatic deposits in one of the vertebrae (lamina of third dorsal).

the service of Dr. Frank Meara, June 26, and died four months later. The family history was negative and there was nothing of interest in the patient's personal history other than that, ten years previous to admission, he had sustained an attack of plumbism followed by stiffness of the wrist, hip and knee joints that disappeared in the course of the succeeding two months.

At the time of admission the patient complained of severe burning pains in the back that had been present for three weeks. Three days

before admission these pains became so excruciating that he was unable to stand and had to give up work and take to his bed. In addition, he complained of pain in the upper sternal region on swallowing. At the time of admission, it was noticed that the entire dorsal spine was rigid and that this rigidity was increased by movements of the trunk. Two weeks later the patient could not move the body below the waist. The superficial reflexes were abolished, while the knee jerks were preserved, and he was insensible to pain up to a girdle point about 5 cm. above the lower border of the ribs. On the following day, there were complete paralysis and anaesthesia of the entire body below the level corresponding to the fifth dorsal segment. The patient now complained of no pain except when the head was moved, at which time pain was severe and was referred to the region between the fourth and sixth

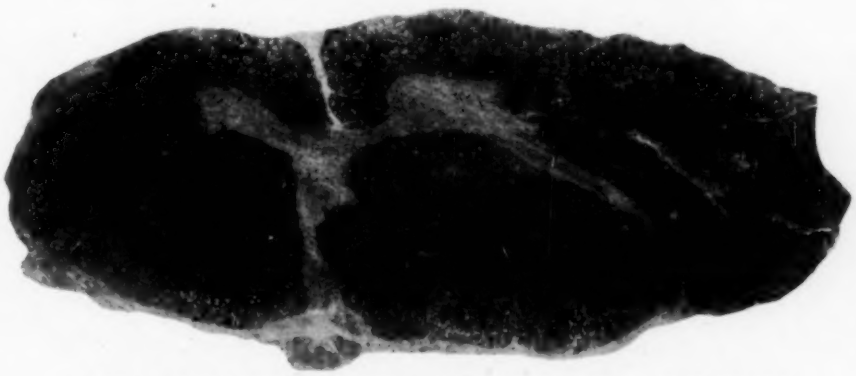

Fig. 7.—Marchi preparation from the spinal cord corresponding to the area of compression, showing the mechanical effects of crushing and the widespread degenerative changes in the nerve elements.

dorsal vertebrae, where there was also tenderness on pressure. The knee jerks had disappeared and there was a bilateral Babinski. The patient suffered from retention of urine and was incontinent of feces.

The spinal fluid was yellowish in color and contained thirty cells to the cubic millimeter. Examination of the urine revealed nothing of note in the present connection. The Wassermann reaction was negative.

July 16, the spinal column was opened from the fourth cervical to the second dorsal vertebra, and a tumor was removed from the dura mater corresponding to the region between the sixth cervical and the second dorsal vertebra. The growth measured 6x2½ cm. and pressed on the dorsal cord. In the succeeding three months the patient gradually failed, bed sores developed and death occurred October 25.

Except for pain on swallowing, the patient at no time during his stay in the hospital exhibited signs or symptoms referable to pressure in the anterior mediastinum.

REPORT OF NECROPSY

The body was that of an extremely emaciated man, 58 years of age. In the upper portion of the back, lying in the long axis of the spinal column, was a surgical incision 22 cm. in length. Over the sacrum and on the calves of the legs and heels were gangrenous bed sores.

On opening the thorax, a mass came into view corresponding to the position of the thymus. The mass measured 10 x 8 x 3 cm., and was roughly triangular in outline, with the base upward. The growth was milk-white in color and of cartilaginoid consistence, and was attached to the border of the left lung for a distance of about 6 cm. On section, in the center of the tumor, there was a yellowish, cheesy area of softening. Scattered through the left pleura were a few firm nodules, varying in size from ½ to 1 cm. The tissue composing these nodules was quite similar to that of the mass in the thymic region. On section, both lungs were studded with nodules of similar description, varying in size from 1 to 2 cm. In some of the larger nodules were yellowish gray areas of degeneration, and others showed grayish white radiating bands of fibrous tissue. The distribution of these nodules was roughly symmetrical throughout both lungs.

The heart was not displaced. The transverse portion of the arch of the aorta passed immediately behind and was partially included in the tumor lying in the thymic region. The aorta itself, however, was not infiltrated.

The laminae of the vertebrae, from the fourth to the eighth dorsal, had been removed. The lamina of the third dorsal vertebra contained dense white tumor tissue lying in the substance of the bone. The spinal cord corresponding to the excised laminae was compressed and there was a wartlike growth, whitish in color, on the posterior and external surface of the dura at about the level of the third dorsal vertebra.

The lungs showed, in addition to the nodular metastases, numerous minute grayish points corresponding to miliary tubercles.

In the small intestine were several ulcers with rounded, undermined edges and fibrous base that varied in size from ½ to 1½ cm.

Anatomic Diagnosis.—Malignant tumor of thymus with metastatic nodules in the pleura of the left lung, in the parenchyma of both lungs, in the lamina of the third dorsal vertebra, and in the dura of the dorsal cord; compression of dorsal cord; miliary tuberculosis of lungs; ulcerative tuberculous enteritis; emaciation.

Histologic Examination.—The reticulum of the parent tumor in the thymus region consists of rather broad bands of dense, poorly staining connectiv: tissue scattered through which are moderate numbers of delicate cigar-shaped nuclei. The fibrous ground substance divides and subdivides in such manner as to segregate the tumor cells into groups which vary markedly both in size and shape. The tumor cells themselves present wide differences in morphology and in their relationship to one another. Perhaps the commonest variety is a rather small cell with a deeply chromatic homogeneous nucleus. Under ordinary magnification, this cell appears to be rounded and of about the size of a lymphocyte, but when viewed under the oil immersion lens with properly diminished illumination, it is seen to be irregularly rounded or even polyhedral and to possess a quantity of pale, smooth, or finely granular cytoplasm. This appears to be the cell of origin of the tumor and it may be traced through various transitional stages until it acquires a flattened form (Fig. 1). These flattened forms give rise to still larger cells of extremely variable morphology. The smaller cells may be seen in abundance scattered through the connective tissue frame-

work as islands or streak-like collections of different shapes and sizes (Fig. 1, upper half). In other instances the small cells are found in company with larger, flattened cells which are arranged in solid groups consisting of small oval or rounded collections, sinuous bands, or intercommunicating cords, or as large islands of variable contour (Figs. 1 and 2). In the large islands the flattened cells sometimes display a tendency to arrange themselves in small concentric whorls (Figs. 2 and 3). In other islands of flattened cells, groups of small cells are to be noted lying in the center or at the periphery (Fig. 1, lower half). In still other instances, the islands of flattened cells present marked variations in the shape and size of the individual elements (Fig. 4), prominent among which are huge hyperchromatic bodies (Figs. 4 and 5), some containing a single nucleus, others a nucleus that is lobulated, still others multiple nuclei. In some of these giant cells, evidences of keratinization are to be observed. Lying among and around the giant cells are somewhat smaller cells that present almost every conceivable variation in shape. A noticeable feature of the large, irregularly shaped cells, as a whole, is that they show attempts to become arranged in whorls and thus to present a certain resemblance to epithelial pearls or to Hassall's bodies (Fig. 4).

Microscopic examination of the metastatic deposits in the vertebral column (Fig. 6) shows the presence in the marrow of tumor tissue of the same general appearance as that of the parent growth in the thymus, and the same is true of the secondary nodules in the dura, pleura and lungs.

Marchi preparations (Fig. 7) show degeneration of the entire thickness of the spinal cord at the level where the tumor pressed on it. Sections of the cervical cord above the site of the tumor show complete degeneration of the column of Goll, including the cornucommissural bundle, the dorsal and ventral spinocerebellar tracts, the lateral and mesial spinothalamic tracts. Below the level of the lesion the lumbar segment shows degeneration in the crossed and direct pyramidal tracts and the scattered fibres of the Deiterospinal tracts. The sacral segment shows degeneration in the crossed pyramidal tract and a few fibres in the anterior ground bundle, probably Deiterospinal.

Microscopic examination of the other organs of the body showed nothing worthy of record in the present connection.

DISCUSSION

From the clinical standpoint, tumors of the thymic parenchyma, both epithelial and lymphocytic, present certain features of practical interest. First, the lymphocytic tumors outnumber the epithelial by a considerable margin. Moreover, the lymphocytic tumors in the majority of cases occur in individuals under 35 years of age, many of them in children, while all of the few epithelial tumors that have been recorded occurred in persons over 50 years of age.

Second, there are considerable variations in the matter of physical signs. For example, (a) certain tumors of the thymic parenchyma grow expansively in the upper thorax for long periods of time without giving rise to noteworthy signs of pressure. These growths represent a minority, it is true, but that they exist is shown by the complete absence of pressure effects in Rubaschow's case and by the presence of a comparatively trivial complaint in the case that we record, namely, pain on swallowing. (b) Others grow expansively in the upper thorax and are attended by marked signs of pressure—cough, expectoration, dyspnea, cyanosis, and edema, particularly of the right side of the

chest anteriorly and of the corresponding arm, due to interference with the circulation in the innominate and subclavian veins; hydrothorax, ascites, and the like. A subdivision of this group is represented by those thymic lymphosarcomas which grow expansively for a period of months or years, and suddenly terminate life with the picture of acute leukemia, the tumor pouring lymphocytes into the circulation abruptly and in large numbers—the so-called leukosarcoma of Sternberg.[10] As a rule, these growths are attended by pressure symptoms. In one of Sternberg's original cases, however, clinical disturbances are said to have been of only three weeks' duration; yet the intrathoracic tumor was of enormous size and must have been in existence for a period greatly in excess of that suggested by the clinical history. Belonging in this group, also, are those thymic lymphosarcomas which exhibit an extraordinary tendency to bring about symmetrical and diffuse lymphocytic infiltration of the kidneys and massive enlargement of these organs without, however, producing other than slight disturbances of the renal functions. These tumors are usually attended by symptoms of intrathoracic pressure, although in two of Symmers' cases[11] of thymic lymphosarcomas clinical symptoms were present for only three and two weeks, respectively, while in both instances the primary tumors, at necropsy, were found to be immense, so that their origin must have antedated the onset of symptoms by many months.

From these facts it is apparent that primary thymic tumors, whether of the lymphocytic or epithelial type, are inconstant in producing signs of intrathoracic pressure—some of them appear to infiltrate so insidiously that the structures of the mediastinum are enabled to accommodate themselves to the process of invasion, and pressure symptoms, if they arise at all, are negligible in degree; others gradually attain enormous proportions and, suddenly and for no apparent reason, become associated with signs of intrathoracic pressure, followed in a few weeks by death, while still others are marked by pressure signs that arise early and are persistent and most distressing. Finally, primary tumors of the thymus, as exemplified by the case we have described in this paper, may give rise to comparatively mild signs of intrathoracic compression, but, by metastasis, may produce terrible destruction in adjacent extrathoracic tissues.

From the standpoint of malignancy, it is important to observe that the lymphosarcomas and epitheliomas of the thymus gland do not differ essentially from tumors of the same sort occurring in other localities. Wherever it arises, the lymphosarcoma is eminently a growth of regional distribution although, as mentioned, it occasionally displays

10. Sternberg: Beitr. z. path. Anat. u. z. allg. Path. **61**:75, 1916.
11. Symmers: Arch. Int. Med. **22**:237 (Aug.) 1918.

a faculty for bringing about lymphocytic invasion of the blood stream and certain viscera, particularly the kidneys. At other times, it is associated with lymphoid nodules in such organs as the liver, kidney and suprarenal capsule, where, in normal conditions, lymphoid cells are present only in the form of foci so minute as scarcely to attract notice even on microscopic examination. To the unaided eye these nodular "metastases" of lymphosarcomas appear to be well circumscribed but, on microscopic examination, they fade into the surrounding tissues in such graceful fashion as to suggest hyperplasia of pre-existing lymphoid depôts rather than newly formed foci developing from transplanted cells.

In the same way, the epitheliomas of the skin and mucocutaneous junctions commonly exhibit a tendency to remain localized—that is to say, to infiltrate neighboring tissues rather than to metastasize to distant parts. That they do at times become widely disseminated is not, of course, to be denied, but in these circumstances metastasis is apt to occur as a late event.

These facts are of moment as applied to the lymphosarcomas and epitheliomas of the thymus gland, our present knowledge of which is based practically exclusively on evidence derived from the completion of neoplasmic growth as revealed by examination of the body after death. As the attention of physicians becomes more and more focused on the early diagnosis of tumors of the thymus gland, it is not too much to hope, perhaps, that at least some of these growths may be discovered before neighboring tissues have been irreparably damaged. The preponderance of lymphoid growths of the thymus and the known effects of radiation on lymphocytic tissues suggest that the use of the roentgen ray might be beneficial in the treatment of tumors of this type. Also it is conceivable that greater attention to the interpretation of symptoms of pressure in the anterior mediastinum, coupled with such diagnostic aids as the roentgen ray, might sometimes lead to detection of thymic tumors sufficiently early to permit of their enucleation before they have progressed too far.

BETELNUT CHEWING AND ITS EFFECTS, INCLUDING CANCER OF THE MOUTH

ALLER G ELLIS, M.D.
BANGKOK, SIAM

Medical literature contains many references to cancer of the mouth in betelnut chewers. This sequence of events is cited in illustration of malignant growth developing as a result of chronic irritation (injury) of tissue. Statements regarding this subject are based largely on observations made in India, Africa and some of the islands in the Pacific Ocean. The receipt in the pathological laboratory of Siriraj Hospital of specimens of cancer of the lip from persons using betelnut, suggested an investigation of this question in Siam where the habit is exceedingly prevalent. It was soon found that the scarcity of systematic records in this country makes an extended statistical study of cancer impossible, but, as in other countries, very much that cannot be reduced to exact figures may be learned. The subject was accordingly taken up in a somewhat broader way, and this promptly led to the realization that another of its phases is of more practical importance than is its relation to cancer.

As a part of this study, I sent a questionnaire to twenty-five physicians, including those at the various Mission stations, and the replies thereto represent all parts of the kingdom. Much of the data contained in this paper was furnished by these colleagues, for whose aid I am very grateful.

Except in the larger cities, nearly all adult Siamese chew betelnut. Exceptions in the cities occur among those who have been in foreign countries or who have attended advanced Siamese or foreign-taught schools. In some of the provinces there is said to be a decrease in the habit in the younger generation, but this is not in all instances clearly demonstrable; in Bangkok it apparently is true. It is said that habitual users in the south chew much greater quantities than do those in northern Siam. Chewing is more nearly universal among women than among men and, in general, women chew more of the substance. Some of the large numbers of Chinese here are addicted but the majority are not; among them, women chewers are less numerous than are men.

Betelnut chewing, as practised here, is a very complex procedure. In making up what tobacco devotees call a "chew," people use many substances. Of these at least four are of recognized medicinal value, thus contributing to the medical feature of the subject.

A.—THE INGREDIENTS EMPLOYED

1. The leaves of *Chavica betel* or *Piper betel*, commonly called betel leaf (colloquially, plue or ceri leaf). These are glossy green, ovate leaves, from 3 to 5 inches long and from 2 to 4 inches wide; they have a burning, aromatic, bitter taste. They are obtained from a vine that is grown on plantations in the shade of small trees planted for that purpose. The vine is sturdy and grows to from 8 to 12 feet tall. The leaves are ready for use when the vine is 1 year old, and are gathered as they attain proper size for about four years when the plant has to be replaced.

Writers differ as to the essential principles of the leaf. Khory and Katrak [1] say that it contains betel oil and chavicol, the latter obtained by treating betel oil with caustic alkali. The juice of the leaf, they say, is a stimulant, carminative and antiseptic. It is given in cases of flatulence, fetor of the mouth, dyspepsia and colic. They claim that chavicol is a powerful antiseptic, five times stronger than phenol.

Kirtikar and Basu [2] say the leaves are used in India for many purposes, as the juice is aromatic, carminative, stimulant and astringent, and is regarded as a valuable stomachic. For this reason, ancient Hindu writers recommended taking it "early in the morning, after the morning, after meals and at bedtime." In catarrhal and pulmonary affections, the leaves are smeared with oil, warmed and applied over the chest to relieve cough and dyspnea. These writers state that recent analyses by workers in India revealed phenols and terpene-like bodies but failed to detect chavicol.

2. The nutlike seed of *Areca catechu*, commonly called the betelnut palm. The only reason I have found for giving it the latter name is that the nut is chewed with the betel leaf, and from this has acquired the name of betelnut and the tree that of betelnut palm. Literally, there is no such thing as betel "nut" but the name is so common that it would be unwise to attempt to change it. I use the term betel many times in this paper as a shorter word but not with the idea of supplanting the older name. The fruit is nearly two inches in diameter, with a thick green husk and orange colored kernel. This ingredient, including the kernel and the inner layer of the covering, is preferably used fresh, but much of it is preserved by opening and drying.

The kernels are said to contain catechu, tannic and gallic acids, oily matter, gum and several alkaloids, the chief of which is arecoline. Khory and Katrak say the fresh nuts are intoxicating and cause giddiness. (That this is the effect on beginning chewers is common knowledge.) Dried ones are a gentle stimulent, astringent and tenifuge.

1. Materia Medica of India, 1903.
2. Indian Medicinal Plants, 1918.

They increase the flow of saliva, lessen perspiration, sweeten the breath, strengthen the gums, remove bad tastes from the mouth and produce mild exhilaration. They are recommended in cases of intestinal worms and dysentery. In its action arecoline resembles pelletierine or pilocarpin; as a myotic it resembles physostigmin. The powder obtained by calcining the nut is known as areca charcoal and is used as an ingredient of a tooth powder.

Kirtikar and Basu say that chewing the dried nut produces a stimulating and exhilarating effect. They quote Dr. Waring as stating that the claim of its being an anthelmintic is hardly to be supported because intestinal worms (lumbrici) are almost universally present in Hindus and Burmese who chew it. In Borneo the fragrant flowers of the tree are mixed with medicines and used as charms for the cure of many diseases.

3. Black catechu or cutch. This is the extract of the wood of *Acacia catechu* (Siamese sisiet tree). Dey [3] describes it as an astringent and tonic of value in diarrhea, sponginess of the gums and mercurial salivation. It is said to be more powerful than pale catechu from *Uncaria gambier*. This substance appears to be used in the northern part of Siam more than in the south.

4. Root of *Curcuma aromatica* or wild turmeric. This plant is said to contain a volatile oil, resin, starch, sugar, gum and curcumin, a yellow coloring matter. It is used in medicine as a stomachic, stimulant, carminative and tonic. As a masticatory, the powdered root is mixed with lime (paragraph 5) and enough water to make a paste which becomes red in color, possibly due to oxidation.

5. Lime.—This is of the ordinary slaked variety (limestone) or from shells. The use of one or the other apparently depends largely on locality. This substance is mixed with the turmeric and water to form a thick red paste, the usual way, or by some it is used as a powder without the turmeric. The lime is used to counteract the "hot" and astringent taste of the betel leaf.

6. Tobacco.—This is raised in northern Siam. "The leaf is of a peculiarly fine texture. A large portion is used for chewing, mixed with the areca nut and betel leaf. Foreign tobacco is never used in this way."[4]

7. Minor ingredients, such as camphor, cloves, sen-sen, tea leaves, and chestnut tree bark are added by some persons according to their taste. The use of such substances varies to some extent in different parts of the country and appears to be partly a question of local custom.

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3. Indigenous Drugs of India, 1896.
4. Carter: The Kingdom of Siam, 1904.

The chief or staple ingredients, therefore, are betel leaf, areca nut, black catechu, lime-turmeric paste and tobacco. Four of these without including tobacco, have medicinal qualities. In physiologic action three of them are astringent. Other attributes possessed by or claimed for the substances collectively are those of carminative, tonic, stomachic, antiseptic, sialagogue, antidiaphoretic vermifuge and dentifrice. The effect of the mixture when chewed is moderately stimulating and exhilarating. The effect on the beginner is something like that of using tobacco the first time; giddiness and faintness occur but not nausea.

B.—METHOD OF USING

This varies considerably. When lime paste is used, a small quantity is spread on the central portion of a fresh green betel leaf. To this are added the other ingredients, or certain ones only, or none at all. The leaf is then rolled into an elongated cone, about three inches in length and less than one-half inch in diameter at the large end, if the areca nut has not been included. The prepared leaf is then taken into the mouth, followed by the areca nut or tobacco or catechu or all these, and any other desired substance that has not been wrapped in the leaf. Older people without teeth often put all the ingredients into a tube with a movable bottom and mix or rather tamp them together with a plunger. The mass is then pushed out at the top and taken into the mouth.

Actual "chewing" varies with different persons much as it does with tobacco users. Some chew actively and use from fifteen to thirty preparations per day; exceptional cases of persons who use from fifty to eighty preparations daily are reported. Other users are more moderate, the mass often being held for some time between the teeth and the lower lip, either in the midline or toward one angle of the mouth; some keep it more within the cheek. Most people expectorate the large quantity of saliva that collects with the juice, the mixture being a fluid of deep brownish-red color. (On clothing this makes a stain that is removed with great difficulty.) Some persons, especially those who chew unostentatiously, like some users of tobacco, swallow a part of the saliva.

C.—LOCAL EFFECTS OF BETELNUT CHEWING

In the habitual chewer, the lips and tongue and, to a lesser degree, the mucosa of the cheeks are colored brownish-red. Castellani and Chalmers [5] speak of a condition that might be called red or purple tongue which may be puzzling to a medical man newly arrived in the East until he finds that it is due to chewing betel. They say the pigmentation slowly disappears when the natives discontinue chewing.

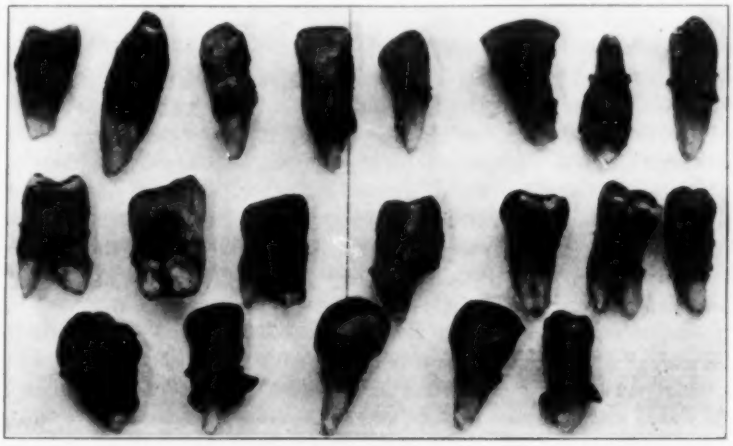
5. Manual of Tropical Medicine, 1919.

The tongue may remain smooth but often it becomes very dark in color and the margins dry and rough or even cracked. I have seen a considerably roughened tongue in a girl, aged 13 years, who had been chewing for only one year. The lips of some chewers become very rough. It is not uncommon to see women with the mucosa of the lower lip (which may be thickened and everted) entirely denuded and roughened by quite deep furrows. Such mucosae are dry rather than inflamed and exudative. The upper lip is affected to a much lesser degree. The entire mucosa of the mouth undergoes a more or less marked change which may, perhaps, appropriately be described as tanning.

The teeth become very dark brown or almost black in color. This is due to a deposit of the colored lime (so-called calcium concretion) which collects on the teeth. This begins on or near the extremity of the tooth and finally covers over the entire crown, unless it be the points of cusps. Actual "staining" of the teeth does not seem to occur; at least in those teeth which I have examined the enamel is intact and perfectly white when the concretion is removed. As a rule, the concretion is very closely adherent and has to be removed with a knife. On some teeth the deposit has a dull, rough surface, but on others, or on parts of them, it is smooth and has a shining luster like polished ebony. The thickness of this deposit varies. On the biting surfaces it is usually quite thin if the teeth are in actual use. If they do not meet because the opposing ones are out or are loose, it may become very thick. Occasional teeth are found with a deposit on the end thicker than the length of the crown itself.

On the sides of the teeth, the deposit, in many instances, becomes quite thick. Occasionally, the space between contiguous teeth is filled in, converting two or more into a solid mass. If the gum separates from the neck of the teeth and they become loose, the deposition continues downward on the root, as it is gradually exposed by atrophy of the alveolar processes and consequent lessening in depth of the socket. In this way, it finally covers a large part of the root. Measurements of a few teeth extracted when almost loose enough to fall out will give an idea of the surprisingly short portion of some roots left uncovered by concretion. The first number given is the length of the entire tooth; the second number is the length of the uncovered portion of the root from the lower edge of the concretion to the tip. A. 20 mm., 5 mm.; B. 28 mm., 6 mm.; C. 27 mm., 2 mm.; D. 25 mm., 10 mm.; E. 18 mm., 3 mm. (See illustration). All these are teeth with single roots, incisors and canines. One molar has a concretion covering the external surface of one root to its very tip and forming a mass 5 mm. broad at the extremity. The other two roots are about half covered; the entire crown is encased. The larger part of this particular concretion is gray in color.

The lower border of the concretion on many teeth, like those described, or even when it extends only to the neck or slightly below, is rough. Some extending downward on the roots taper to almost knife-like thinness. Most of the latter for the lower few millimeters of their length are 1 or 2 mm. distant from the tooth. Just what was in this space between tooth and concretion while the latter was being formed is not entirely clear; the opinion of dentists is that it was a portion of the receding gum which had kept close to the tooth as it was carried downward by atrophy of the alveolar process beneath and pressure by the concretion above. I am unable either to verify or



Teeth (from different persons) which became loose and were extracted. Those in the upper rows with the cusps showing white had opposing teeth; those with entirely dark crowns, as in the lower row, had no opposing teeth and show very thick deposit on the ends as well as on the sides. Note the very short portion of some roots left uncovered by concretion. Teeth like those on the extreme right and the second tooth from the left in the lower row lend weight to the view that concretions press down the gum. The tooth on the extreme right of the middle row and the tooth on the left in the lower row have spaces between the concretion and the root, this not being well shown in a side view.

The light mark on the fourth tooth in the middle row was made in an attempt to saw the tooth lengthwise; the enamel under the concretion proved to be harder than the saw. The concretion on the second tooth from the left in the middle row is the only one not colored; it looks like fine gray limestone. From the light areas on the third and fourth teeth in the upper row, the concretion was scraped away with a knife.

disprove this. These projecting rough concretions must be a continual source of irritation to the surrounding tissue. They explain very definitely the statement of one physician that it is impossible to cure pyorrhea and clear up foul gums until these concretions have been removed.

The unanimous statement of physicians and dentists (and the laity) is that betel chewing prevents decay of the teeth. This is abundantly substantiated by the conditions found. One says "Actual decay of the teeth is practically unknown among these people." The covering of the teeth by the dense, polished, intimately adherent concretions apparently protects them. Whether the action of the chewed substances themselves on teeth unprotected by deposit prevents caries is difficult to say because such teeth in chewers are not numerous. Some believe that this is true, and that moderate chewing without deposit is beneficial to the teeth. I do not know that this has been demonstrated in such a way as to warrant the positive statement. However this may be, the statement can be made that so far as caries is concerned, betel chewing, as popularly indulged in, is a preservative of the teeth. I believe this is the only favorable statement that can be made about it. If used to a limited degree as a dentifrice only, with frequent removal of the lime from the teeth, the gums and teeth might be benefited, although I cannot conceive of any one using it for this purpose alone.

Opposed to this preservative action as regards caries is the fact that the loss of sound teeth is very common among betel users. That pyorrhea is prominently implicated in this is indicated by these statements in my reports: "Pyorrhea is very prevalent; the teeth are usually loosened"; "pyorrhea practically universal"; "teeth are loosened by pyorrhea and retraction of gums"; "very little decay, very much loosening"; "chronic condition of pus discharging sockets for years"; "dental caries very rare. Loosening of the teeth very common"; "a native may have lost all his teeth by 50 years of age or less"; "little or no decay of the teeth. The gums receding the teeth project and may be removed with the fingers"; "a distinct preservative from decay, even if a tooth is badly broken, but teeth are loosened by pyorrhea and retraction of gums"; "many people at 40 are quite devoid of teeth."

Some of these statements are not as definite as could be desired, but the only conclusion to be drawn from them as regards pyorrhea is that this condition is exceedingly common. Quite dissimilar to this are the findings of Dr. H. R. Day, dentist at the Chulalongkorn Hospital, who states that of the many loose teeth extracted by him, not more than 10 per cent. exhibit pyorrhea. For this discrepancy in findings I think there can be invoked at least three reasons: (1) The well known difference of opinion among physicians and dentists as to exactly what constitutes pyorrhea alveolaris. How much separation of gums, how much pus, how much looseness of teeth, etc., must be present as a basis of diagnosis? (2) Lack in many cases of special examination of persons' mouths with the points raised in this paper definitely in mind. (3) The possibility that in some instances of very loose teeth with very

shallow root sockets due to atrophy of the alveolar processes, pyorrhea may have been present earlier and disappeared as more thorough drainage was established by this lessening in depth. For this reason, I do not believe that the question of pyorrhea can be settled by a study of these very loose teeth. This view has been further strengthened by my observation of earlier cases, although relatively few in number. Of twenty-seven persons whom I have examined, six had distinct pyorrhea and eight more a condition that might by some be termed an early stage of that process, but which conservatively had best be excluded. Four of the six who had pyorrhea, and five of those who did not, had one or more loose teeth; all of the latter series were in those with possibly early pyorrhea.

The importance of this point is not whether pyorrhea loosens teeth, because it does, but what percentage of the teeth becoming loose in betelnut chewers is the result of pyorrhea, and what percentage is the result of other factors strongly emphasized by some physicians and dentists? Among these factors are: (1) The mechanical pressure of concretions on the gums and alveolar processes, causing the latter to atrophy and the former correspondingly to recede. According to this view, the sockets progressively become shallower and the teeth become loose because they are deprived of support. (2) Shifting of the line of pressure by opposing teeth from vertical to diagonal because of concretions forming unequally on them. The direction of this secondary line of pressure varies from time to time as the concretion is building, and finally the tooth is loosened.

The limited number of cases I have studied has given me the impression that loosening of the gums from the teeth and infection between the two is quite common before concretions are large enough to cause notable pressure. In occasional cases, there has been shrinking and separation of the gums when the deposit extended only half way from the extremity of the tooth to the gum. In those cases in which there is a thin layer of deposit extending beneath the level of the gum, the inference must be that separation of the gum first occurred or the deposit could not be between it and the tooth. In this connection the statement of Tomes [6] made years ago is pertinent. In discussing the relation of tartar to pyorrhea alveolaris (of course, in people not chewing betelnut) he says, "One of the earliest indications of the advent of this state of things is a thickening and rounding of the edge of the gum, which ceases to be closely adherent to the neck of the tooth." He states that the dark tartar found between the edges of the gums and the teeth could not have gotten there until the gum had begun to peel away from the necks of the teeth. I have received

6. Dental Surgery, 1887.

this impression regarding the thin lime concretions seen in many cases of loose gums. However, it has not been my good fortune to see advanced cases with teeth hanging only by the tip, and with massive concretions like those shown in the accompanying illustration. Possibly, in those cases the mechanical feature of pressure would appear much more prominent.

I believe that the exact mechanism of loosening of the teeth (the same as the incidence of pyorrhea) cannot be determined from a study of advanced cases alone. These questions as to the frequency and effect of pyorrhea and the other possible factors in loosening the teeth can be solved only by long and careful study of many cases of various degrees (especially the early cases) of involvement, including repeated examination of the same persons at intervals of months or years. Now we can only say that a large percentage of betel chewers lose their teeth, but that the mechanism by which this loss occurs is not well understood.

The loosening and loss of teeth, from the standpoint of digestion and nutrition, is serious, and if this result of betel chewing can be lessened or prevented, the question is of great practical interest to any country whose people indulge in the habit. Cleaning of the teeth, the same as for those who do not chew, would seem to be of prime importance. I am informed that those who do this thoroughly, with certain preparations, do not so surely loose their teeth. This is a point to be investigated by physicians, and recommendations should be made to users. If the separation of gums from the teeth by the astringent action of the substances used is a primary effect, the way is paved for infection or for absorption of peridental structures, and simple cleansing in itself cannot be of great efficacy in saving teeth. Possibly, some other prophylactic may be of use. If, on the other hand, the loosening is a mechanical process in which the deposit of concretions is the primary factor, frequent and thorough cleansing, to prevent the accumulation of lime, would naturally be the indication. A comprehensive study of the whole subject appears very desirable.

C.—CONSTITUTIONAL EFFECTS

As to digestive or other constitutional effects of betel chewing per se, most observers agree that these are slight. A few observers speak of fairly common digestive disturbances among the Siamese people (in the opinion of one—starch indigestion), and of a suspicion that this is an indirect effect of the substances chewed or of the saliva that is swallowed. (It would be interesting to note whether this occurred especially in persons with loose and tender teeth. Constipation as an effect is mentioned by one observer only; some say this is a noteworthy condition only in old people who do not take exercise. Dr. R. W. Mendelson, acting medical officer of health of Bangkok, states that the

observation of more than 40,000 patients in their hospital clinic has led him to believe that betel chewing has no untoward digestive effect; his findings in the gastro-intestinal tract postmortem have confirmed that belief. My own experience in nearly 200 postmortems here has led to a similar conclusion. Although no microscopic study has been made of the stomach and intestines in all cases, the gross appearances certainly have not been suggestive of disease. Of course, these negative findings would not exclude the possibility of clinical manifestations.

Rho [7] says that the use and misuse of the elements employed in chewing may appear unappetizing, but damaging effects do not follow. Castellani and Chalmers state that in the young the habit may possibly be the cause of diseases of the heart and nervous system. Most of my correspondents say that their observations have not led them to believe that betelnut chewing causes any untoward digestive or nervous or circulatory symptoms.

As to the general effect on the body of pyorrhea as an infection, much might be said. I do not intend to discuss that problem in any detail. However, it is an infection, and an infection at any point in the body can exert an influence elsewhere. One physician writes: "There is much so-called rheumatism in this locality. Some of it is without doubt gonorrheal arthritis. I am convinced, however, that more than half the number of cases are from other causes, and the pus foci around the loosened teeth are open to suspicion." Whatever may be said, in general, about pyorrhea alveolaris applies to that condition in betel chewers.

D.—THE BACTERIOLOGY OF BETELNUT CHEWING

This study early led me to note the discrepancy between the claim of antiseptic properties for the betelnut mixture and the condition of the mouths of users as described by my correspondents and seen by myself. Theoretically, the mixture employed should be, at least, mildly antiseptic; practically, foulness of the mouth and suppuration are very common. This suggested a bacteriologic study of the mouths of betel chewers. With the permission of Director Luang Ayura Bhatt, examinations were made of twenty patients in the medical, surgical and obstetric wards of Siriraj hospital. The results were so uniform, that it seemed unnecessary to examine a greater number to determine what we wished to know. In this work I was ably aided by Khun Traikisayanukara and Nai Thieb, and it is a pleasure here to acknowledge their help.

The study included a general inspection of the mouths and gums and teeth for decay and looseness. Inoculations were made from the

7. Mense: Tropenkrankheiten, 1914.

margin of the gums between the teeth or from pockets around loose teeth. Spreads were also made for staining, and in fifteen of the cases enough exudate was obtained from the gums or around the teeth to make fresh specimens for microscopic study. From six of the persons a specimen of the red colored saliva was obtained in sterile Petri dishes.

The results of these examinations may be summarized thus:

Persons examined, 20.
Sex: Women, 15; men, 5.
Age: From 13 to 54 years.
Condition of mouth (gums): Clean, 5; moderately foul, 6; very foul, 9.
Lower lip: Cracked, rough and dry, 3.
Gums: Shrunken (contracted) to varying degrees, 20; loose from one or more teeth, varying degrees, 17.
Teeth: Entirely black, 19; bases not black, 1; one or more loose, 9; decayed, one molar.
Pyorrhea alveolaris: of various degrees, 6; probably beginning, 8.
Amebae (Endameba gingivalis): In 9 of 15 fresh specimens.
Polynuclear leukocyte exudate around teeth, 17.
Bacteria: 1. Spreads from gums, cocci in 20; bacilli in 20; spirochetes in 20; fungi in 1.
2. Inoculations from gums, positive in 20; cocci in pure culture in 6; cocci and bacilli in 14; streptococcus in 1; pneumococcus in 1; cocci predominant in 12. Dominant coccus, *Staphylococcus pyogenes aureus.*
3. Saliva (six specimens): Growth from 6; cocci in 3; cocci and bacilli in 3.

From this it will be seen that the mouths of betelnut chewers possess a rich bacterial flora, not exceeded by those (even with pyorrhea) who do not chew. No special attempt was made to isolate and identify the various organisms because an intensive study of the varieties was not an aim. One object was to determine if bacteria, spirochetes and amebas, thrive in the mouth in the presence of the substances chewed and kept within it almost constantly by the users of betelnut. This surely can be answered in the affirmative. Cocci were present, often in great numbers, in every case. The bacilli, also present in every case, were of various types, including some of the large nonpathogenic forms. Spirochetes (*S. dentium* and *S. buccalis*) were present in every case, often in tremendous numbers. Amebas were present in more than one-half the specimens examined for them. These findings do not indicate antiseptic properties of betelnut. A second point of inquiry is really only a variation of the first, namely, has the red colored saliva of the chewer any antiseptic or bactericidal properties. As the saliva and juice collect in the mouth, the mixture is supposedly contaminated by the bacteria on the gums and around the teeth. This supposition was shown to be correct by the abundant growth obtained from inoculations of all six specimens.

In connection with the sixth specimen, the question arose as to what would be the effect on the contained bacteria of continued presence in this juice. Inoculations were made from it after the lapse of twenty-

four, seventy-two and ninety-six hours. Each culture gave an abundant growth of cocci and bacilli, the same as developed from inoculation immediately after the specimen was obtained. This showed that these bacteria had lived in the mixture for four days, apparently with undiminished powers of growth. During this time, evaporation had occurred, and at the end of the ninety-six hours the specimen was only a mass of dry reddish granules. This result indicated that the betelnut mixture possessed no bactericidal properties. To support this conclusion, saliva was obtained from five other chewers. Each gave a growth of cocci and bacilli when obtained and also twenty-four and forty-eight hours thereafter. Two cultures had then developed molds and could not be used further, but the remaining three cultures gave a similar bacterial growth from inoculations made after the lapse of ninety-six hours.

To still further test the betelnut, a "chew" was purchased and macerated in a sterile mortar. Inoculations from the expressed juice remained sterile. Enough sterile water was added to make a thick red fluid and some of this was poured over an agar slant and some into a tube of bouillon. Both were proved sterile and then the agar slant was inoculated from one of the specimens of saliva; in twenty-four hours the red surface showed a heavy growth of bacteria. This betelnut-agar tube was kept for one week, part of the time in the incubator, part of the time at room temperature, and then an inoculation was made from it; that the bacteria were still living was shown by an abundant growth. A spore forming bacillus had also developed at one point.

The tube of bouillon colored red by the betel juice was inoculated, and in twenty-four hours was made turbid by a growth of bacteria similar to those placed in it. The turbidity increased the following two days, and six days later the bouillon gave a positive culture, proving that the bacteria had lived that long in the betelnut bouillon.

From these studies of persons' mouths and of betelnut, we reached the conclusion that if antiseptic properties are possessed by any of the ingredients used in betel chewing, such properties are not manifested in a demonstrable way in the mouths of chewers or in the expressed juice of the substances employed. This is in complete accordance with the clinical findings of frequent pyorrhea, which theoretically should not occur if the substances chewed were inimical to the growth of bacteria. Betelnut chewing causes retraction and separation of the gums from the teeth and the deposit of concretions. The first certainly and the second probably furnishes physical conditions favorable to peridental infection; there appears to be no actual antiseptic nor bactericidal action of the substances to prevent such infection occurring.

E. RELATION OF BETELNUT CHEWING TO CANCER OF THE MOUTH

That this habit causes chronic irritation of the lips and mouth which predisposes to malignant epithelial growth is a quite common belief, though some writers are indecisive in their statements. Coplin [8] says "Persistent or long continued irritation seems to favor the development of tumors belonging to the epithelial group"; as one example of this is cited epithelioma due to chewing betelnut. Ewing,[9] in discussing this question, says: "Nevertheless, it is clear, as in Kangri-oven and betelnut cancer of African natives, that local and general predisposition may be ignored, since the tumor develops wherever the irritant is effectively applied." Castellani and Chalmers, in speaking of the betelnut chewing habit in the East, take a rather neutral ground by stating that "the irritation may be the cause of the commonest cancer of old people in these parts." Scheube [10] comments thus: "The frequency of cancer of the mouth (lips, mucosa of cheeks, tongue, jaw) in British India is referred to the chronic irritation of betelnut chewing."

During my seventeen months in Siam, I have studied microscopically three specimens of cancer of the lip sent from the operating room of Siriraj Hospital. All were from betelnut chewers, although one, a Chinese man, is recorded as chewing betelnut moderately and smoking Siamese cigarets almost constantly; this case, therefore, might seem to be partly a "smoker's" cancer as well as a "betelnut chewer's" cancer. In this case, portions of both lips were removed, the lower presenting a warty elevation 2.5 cm. long, the upper a grayish, smooth, slightly elevated area. The growth on the lower lip proved to be largely a papilloma with early carcinomatous infiltration at the base; the upper lip presented simply thickening of the epithelium with hyperkeratosis. A second specimen was a warty growth, 2 by 1.2 cm., on the lower lip of a siamese woman, aged 47 years. This also was a papilloma with at points a beginning squamous cell epithelioma. The third specimen was from a Siamese man, aged 54 years, who had a rough, slightly elevated growth on the lower lip,—microscopically a papilloma with early carcinomatous extension.

It will be noted, that all three specimens were advanced or distinct papillomas, with only early, but also distinct, carcinomatous transformation, the so-called papillary cancer. This type of growth appears to be quite frequent in this country in connection with the skin and the mucocutaneous borders. That is, there is an extensive papillary growth preceding actual malignant extension, although presumably the latter

8. Manual of Pathology, Ed. 5, p. 306.
9. Neoplastic Diseases, p. 460.
10. Die Krankheiten der Warmen Länder, p. 842.

finally occurs in all of them. From the clinical standpoint, surgical removal, even when the growth is large, should result in a large percentage of cures because of the slight infiltration by the tumor.

In addition to these three operative cases, two persons with cancer of the mouth have within the past few months presented themselves at the hospital outpatient department. One of these I saw. The patient was a man, aged 61 years, who had chewed betelnut since he was a boy, holding the mass in the left cheek. His teeth had loosened and all fallen out before he was 30 years old. The tumor was an extensive one, involving the left cheek and jaw, and presenting on the external surface as a mass 3 cm. in diameter. The other case was one of an ulcerated tumor of the cheek and jaw in an elderly woman. It will be noted that two of these five subjects were women.

Regarding the frequency of tumors elsewhere in the body, the following statement can be made: During the period in which the above three specimens of cancer of the mouth were removed, there were sent from the hospital operating room thirty-two other tumors, of which twenty were malignant and twelve benign. The postmortem service for the same period yielded eleven tumors, eight malignant and three benign.

Through the courtesy of the director, Colonel Phra Sakda, I am enabled to give the statistics of cancer in the Chulalongkorn Hospital of Bangkok for the past six years. They are as follows:

Total number of cases of cancer operated on in hospital, 102.
Sex: Men, 55, or 54 per cent.; women, 47, or 46 per cent.
Cancer of lower lip, 52 cases.
Cancer of tongue, 2 cases.
Cancer elsewhere in body, 48 cases.
Sex, cancer of lip: men, 29, or 56 per cent.; women, 23, or 44 per cent.
Sex, cancer of tongue: men, 1; women, 1.
Sex, cancer elsewhere in body: men, 25, or 52 per cent.; women, 23, or 48 per cent.

From this it will be seen that cases of cancer of the lip and tongue comprised more than one-half the total number, an unusual proportion. Virtually all the patients, men and women, had chewed betelnut for many years, the average age being 48 years. Colonel Phra Sakda regards as very significant the fact that in all cases of cancer of the lip, the lower lip was involved, this being the one most injured by betelnut. There are also very few cases of cancer of the tongue, the organ often affected in smokers in other countries.

To me a most suggestive point is that of sex. Statistics from countries in which the people do not chew betelnut show rather uniformly that at least 90 per cent. of cancers of the lip are in men. In this series of cases of cancer in betelnut chewers, 56 per cent. were

in men and 44 per cent. in women, a very striking difference. Of course, this does not prove that the 34 per cent. increase above the average for women was due to betelnut chewing. But to what other factor shall it be ascribed? These statistics appear fully to justify the opinion of Colonel Phra Sakda that betelnut chewing is a predisposing cause of cancer of the lip.

As to the frequency of cancer of the mouth in betelnut chewers throughout Siam, the accompanying table summarizes the findings and opinions of a number of physicians in various parts of the country. Most of the figures given are admittedly from memory instead of actual records, and are to be taken in their general significance rather than as accurate statistics.

FREQUENCY OF CANCER OF MOUTH IN BETELNUT CHEWERS IN SIAM

Physician	Years of Observation	Cancer of Lip	Cancer of Cheek	Cancer of Tongue	Cancer of Mouth in Nonchewers	Does Betel Chewing Tend to Cause Cancer
1. E. C. C.	12	5	5	1	None	No
2. C. H. C.	16	None	None	None	None	No
3. W. H. B.	6	None	None	None	None	No
4. E. W.	35	2	None	None	None	No
5. L. C. B.	4	3	1	1	None	No
6. E. B. M.	18	25	50	None	None	Yes
7. M. E. B.	3	1	None	None	None	No
8. T. H. H.	34	None	None	None	None	No
9. A. E. B.	6	6	1	None	None	No
10. R. W. M.	3	4	6	None	None	No
11. J. W. M.	30	Probably a dozen			None	No
12. G. B. M.	21	About fifteen			None	Yes
13. T. D.	10	About half a dozen			None	No
14. W. B. T.	28	About half a dozen			None	No
15. C. C. H.	20	About half a dozen			None	No
16. P. S.	6	Chaulalongkorn Hospital			None	Yes

The question answered in the table was: "Have your observations led you to believe that betelnut chewing is irritating and tends toward the production of cancer of the mouth?" As thirteen of the sixteen answered this question in the negative, a large majority (81 per cent.) is opposed to the view that betelnut chewing causes cancer. But the opinion of one of the three constituting the minority is based on the Chulalongkorn Hospital statistics, the most detailed and significant of them all. Another of the minority is a dentist as well as a physician, and he has specially noted the changes in the mouths of chewers. The third reports many more cases of cancer than the combined numbers of the thirteen joining in the majority opinion.

In the light of the foregoing, what statement is to be made regarding betelnut chewing as a cause of cancer in the people of Siam? One thing is plain, namely, that cancer of the mouth in betel chewers is not frequent enough to have brought it definitely as such to the notice of the majority of the medical profession throughout the country. Therefore, if there is the relation of cause and effect between the two,

it is not apparent to casual observation but must be demonstrated by a special study of the problem and the gathering of statistics. Does the information presented in this paper furnish such demonstration? I think it does not—because it is insufficient in extent. On the other hand, in view of the lack of accurate records by so many of the physicians, and the very significant statistics of the Chulalongkorn Hospital, it surely cannot be said that the opposite has been demonstrated. I believe that the burden of proof is in this instance on the majority. Whether or not this be a just view, the question cannot be answered finally until there has been, for many years, accurate observation and recording of all cases of cancer of the mouth (and of all other parts of the body) throughout the Kingdom.

SUMMARY AND CONCLUSIONS

1. Betelnut chewing is a well-nigh universal habit of the Siamese people.

2. Decay of the teeth is prevented almost completely, apparently by the deposit of concretions.

3. Neither antiseptic nor bactericidal action on the flora of the mouth is exerted by the substances chewed.

4. Direct constitutional effects of the habit appear to be negligible.

5. Betelnut chewing leads to chronic changes in the mucous membrane of the mouth, recession of the gums, pyorrhea alveolaris, deposit of lime concretions on the teeth, atrophy of alveolar processes and loosening and loss of teeth.

6. The exact way (or ways) in which teeth are loosened is not well understood.

7. As the loss of teeth appears to be the most serious result of betelnut chewing, a thorough study of its mechanism and prevention is eminently desirable.

8. Betelnut chewing does not frequently cause cancer of the mouth, but there are reasons for believing that, in an as yet undetermined percentage of cases, it does lead to this result.

CAROTINEMIA

REPORT OF A CASE IN AN ADULT

GEORGE DOUGLAS HEAD, M.D., AND REUBEN A. JOHNSON, M.D.
MINNEAPOLIS

Under the term carotinemia, Hess and Myers [1] described the occurrence of a peculiar yellow discoloration of the skin of the body with the presence of carotin, the characteristic pigment of carrots, in the blood of children fed on a diet containing carrots. We wish to report a similar occurrence in an adult, a case in which we were able to establish the casual relationship between diet and pigmentation, and to identify the offending pigment chemically, in a relatively large amount, in the blood.

The yellow pigment carotin is not confined to carrots. It is included with xanthophylls under the general term carotinoids. They are widely distributed in nature, often in close association, and can be separated from each other on the basis of solubility. The yellow color of milk fats, corpus luteum and egg yolk, is due to carotinoids which are sometimes called lipochromes and again, lutein. Among human vegetable food stuffs they are found especially in spinach, lettuce, oranges and carrots. Palmer [2] and his associates have done splendid work in clearing up the problems of carotinoid pigmentation. They have shown conclusively that the natural pigment of egg yolk, body fat and blood serum of the hen is physiologically identical with the carotin and xanthophylls of plants, with the latter present in far greater proportion. In the cow, the coloring of milk fat, body fat, skin secretions, corpus luteum and blood serum is similarly due to carotinoids, but in contrast to the hen, the carotin is in excess with only traces of xanthophylls. They were able to produce almost colorless milk fat or egg yolks by feeding a diet poor in the respective characteristic pigment. Fat is the storehouse of carotinoids and the absence of these pigments explains the colorless fat of swine, sheep and goats. There is, therefore, a wide variation among species in their behavior toward these pigments. Analysis of the fat of human milk showed tinting with both carotin and xanthophylls in nearly equal proportions. They concluded that the pigment of human fat is, no doubt, identical with the pigment of human milk fat.

With the exception of the article of Hess and Myers and an editorial [3] reference, we have found no description in the English litera-

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1. Hess, A. F., and Myers, V. C.: Carotinemia: A New Clinical Picture, J. A. M. A. **73**:1743 (Dec. 6) 1919.
2. Palmer, L. S., and Eckles, C. H.: J. Biol. Chem. **17**:191, 211, 223, 237, 245 (March) 1914; Palmer, L. S.: Ibid. **23**:261 (Nov.) 1915; **27**:27 (Oct.) 1916.
3. J. A. M. A. **74**:32 (Jan. 3) 1920.

ture of a case presenting pigmentation resulting from a diet rich in carotin. A number of articles have appeared in the German literature, especially by pediatricians, during the period of the war when food shortage necessitated a change to a more vegetarian diet. Kaupe [4] observed, in a childrens home, a large number of otherwise healthy children, who showed a marked yellow pigmentation of the skin of the face. Carrots were present in the diet of all those affected but not all the children who ate carrots showed the discoloration. When carrots were withdrawn from the diet, the color disappeared and could be brought back by adding carrots to the diet again. The observations of Kaupe were confirmed by Stoelzner [5] who had seen many similar cases which he called pseudo-icterus. Klose [6] also reported cases of carrot pigmentation, and thought that well nourished children were more prone to the affection, probably dependent on the affinity of the fats for carotinoids. Many years ago, following the use of carrot soup, Moro [7] had seen a yellow discoloration of the skin develop. The actual presence of carotin in the blood was not established by chemical tests in any of the above reported cases.

The condition is not confined to the early years of life. Umber [8] used the term "Diabetische Xanthose" to describe cases of marked yellow skin pigmentation which he had seen in diabetics. He had seen fifteen cases in all and thought that the color varied with the intensity of the disease. The blood, however, contained an ochre gold color, not due to bile, but extractable with ether. The term "Xanthosis Diabetica" was originally used by von Noorden [9] to describe similar cases in diabetics of skin pigmentation varying from a canary yellow to a deep orange gold. In commenting on the reports by pediatricians of pseudo-icterus following the feeding of carrots, Saloman [10] states that it corresponds entirely to the description given by Von Noorden and himself of xanthosis. He had seen many cases, usually in diabetics, but several times in entirely normal people. Spectroscopic examination of the blood serum showed absorption bands identical with those of carotin. Schuessler [11] observed a marked golden

4. Kaupe, W.: Hautverfärbung bei Säuglingen und Kleinkindern infolge der Nahrung, München. med. Wchnschr. **66**:330 (March 21) 1919.

5. Stoelzner, W.: Ueber Pseudoikterus Nach Mohrrübengenuss, München. med. Wchnschr. **66**:419 (April 11) 1919.

6. Klose, E.: Hautverfärbung bei Säuglingen und Kleinkindern infolge der Nahrung, München. med. Wchnschr. **66**:419 (April 11) 1919.

7. Moro, E.: München. med. Wchnschr., 1908, p. 1562; ibid. **66**:674 (June 13) 1919.

8. Umber: Diabetische Xanthose, Berl. klin. Wchnschr. **53**:879 (July 24) 1916.

9. Von Noorden: Internat. Dermatologenkongress, 1904.

10. Salomon, H.: Von Noorden's Handbuch der Pathologie des Stoffwechsels, Ed. 2 **2**:290; München. med. Wchnschr., May 23, 1919, p. 564.

11. Schüssler: Ueber Hautverfärbung durch Mohrrübengenuss, München. med. Wchnschr. **66**:596 (May 30) 1919.

brown (orange) pigmentation of the entire body, without involvement of the sclerae, in three men past middle life who had eaten heavily of carrots as a substitute for potatoes, which were scarce. He made no chemical analyses, and his only explanation for the phenomenon was the excessive ingestion of carrots.

In studies of the pigments found in human blood serum, Hymans van den Bergh and Snapper [12] found serums showing a high lipochrome content. In some of these cases, usually diabetics, a peculiar orange yellow color of the skin, without scleral involvement, was present. Buerger and Reinhart [13] studied a number of cases of xanthosis. They found that there was an increased quantity of lipochrome in the blood serum, that the xanthosis and the parallel increase of lipochrome in the blood came from the food and could be influenced by diet, and that the lipochrome corresponded in chemical and spectroscopic tests to the carotinoid group of pigments which are closely associated with chlorophyll in green plants. Hymans van den Bergh and Mueller [14] confirmed the observation of the dependence of blood lipochrome on the lipochrome content of food. They were able to increase the normally small and somewhat variable quantity of lipochrome in human serum by feeding a diet rich in green leafy vegetables and eggs, and they found increased values in the summer months when vegetables of this type are eaten more freely. The diet peculiar to diabetics leads to an increase of pigment in the blood, and, therefore, to the development of xanthosis. Carotin was found in excess of xanthophylls. They also estimated the lipochrome content of various organs in many diseased states and found a surprisingly high percentage in the suprarenals with lesser quantities in the liver, spleen, fat and blood in the order given. The lipochrome content of the organs seemed independent of the amount in the blood or of any particular disease.

The condition is apparently harmless as no symptoms are ascribed to it. Furthermore, Wells and Hedenburg [15] found no toxic effects in guinea-pigs from intraperitoneal injection and none of consequence on intradermal injection of carotin extracted from carrots, dissolved in olive oil, and used in relatively enormous doses. It is probable, that the carotinoids perform no useful physiologic function in the body.

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12. Hymans van den Bergh and Snapper: Die Farbstoff des Blutserums, Deutsch. Arch. f. klin. Med. **110**:540, 1913.

13. Bürger, M., and Reinhart, A.: Ueber die Genese der Xanthose diabetica, Deutsch. med. Wchnschr. **45**:430 (April 17) 1919; Ztschr. f. d. ges. exper. M. **7**: H 3.

14. Hymans van den Bergh and Müller, P.: Das lipochrome Pigment in Blutserum und Organen, Xanthosis, Hyperlipochromämie, Biochem. Ztschr. **108**:279 (May) 1920.

15. Wells, H. G., and Hedenburg, O. F.: The Toxicity of Carotin, J. Biol. Chem. **27**:213 (Oct.) 1916.

An abnormal pigmentation of the skin, therefore, has been seen to arise from a diet containing unusual amounts of carotinoids. The color is described as varying from a lemon yellow to a rich orange in well developed cases, and can easily be differentiated from jaundice by the difference in color and the fact that the sclerae are not affected. Some portions of the body may be more heavily tinted than others; the palms of the hands, the soles of the feet, the forearms, sternum, the nasolabial folds, cheeks and forehead are especially mentioned in some reports. There is apparently some variation in individuals with respect to the ease with which the pigmentation may develop; possibly being more likely to occur in well nourished persons. When the excess of carotin bearing food is removed from the diet, the skin resumes its normal color more or less slowly. It is reasonably certain that the xanthosis diabetica of the older writers is carotinemia without a correct interpretation having been made. Several observers had previously suspected, from spectroscopic study, the true nature of the serum pigments, but the demonstration of an increase of the carotinoid group of pigments in the blood serum, dependent solely on the same pigments in the food supply in these cases of pigmentation, is a very recent achievement. The condition produces no harmful effects.

REPORT OF CASE

History.—J. B., male, aged 35, bank clerk. Family and previous history unimportant. He was first seen in January, 1919, presenting the classical symptoms of diabetes mellitus which had been present in severe form for about two weeks. He was then on a restricted carbohydrate diet prescribed by his local physician. Physical examination showed nothing of importance aside from moderate emaciation. The urine contained a moderate amount of sugar and acetone bodies. Hemoglobin, 78 per cent. Starvation for twenty-four hours cleared up the glycosuria following which a fairly liberal diet was gradually established without its reappearance. He was taught how to test his urine for sugar and was sent home with instructions to report at intervals for examination.

The next visit was in April, 1919, at which time he called attention to a light yellow tinging of the skin which he and others had noticed. A correct explanation could not be given but a true jaundice was ruled out on the absence of symptoms, bile in the urine and discoloration of the sclerae. Examination of the eye grounds by Dr. Campbell of Minneapolis, showed no fundus changes, and the visual disturbance of which the patient complained was explained as astigmatism. In August, 1919, he was still sugar-free; the Wassermann was negative, and the blood showed a moderate secondary anemia. In November, 1919, he returned feeling well, with a fairly high carbohydrate tolerance and a blood sugar reading of 0.148 per cent. In February, 1920, sugar appeared in the urine with the blood sugar 0.18 per cent., after a fairly large carbohydrate intake.

He had not been seen for about a year when he reported for examination in January of this year. During the entire period since he first presented himself many urinalyses by himself (almost daily) and our laboratory, showed no sugar, except when determining tolerance. Acetone bodies had been consistently absent. He had held his weight and strength and his general condition was excellent. In his correspondence he referred from time to time to the yellow color which he had already called attention to in one of his early visits.

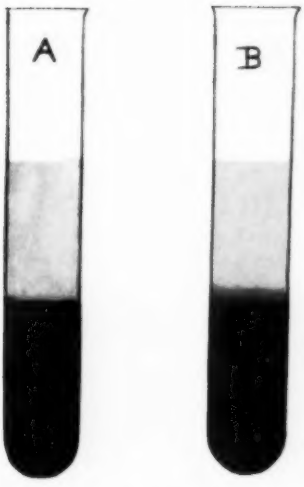
Examination.—His appearance was certainly very striking. There was a high grade, diffuse, generalized, bright orange yellow pigmentation of the skin of the entire body, varying somewhat in its intensity of distribution, being especially vivid in the palms of the hands. The sclerae were normal. There were some dirty brown chloasma-like patches on the skin of the upper abdomen. He stated that the yellow color would last only a few days, sometimes three; his wife might also notice the heightened color and then it would suddenly disappear. This seems to us to be faulty observation as it had been present for many months and did not behave in this manner while under direct observation. He had no abdominal pain and no discomfort whatever but worried constantly about his unusual appearance as he was fearful that it meant some serious derangement. The blood sugar was 0.167 per cent. The urine was free from sugar, acetone, diacetic acid and bile pigments. Blood examination showed hemoglobin, 73 per cent.; erythrocytes, 4,475,000; leukocytes, 5,250. Differential count: polymorphonuclears, 57.5 per cent.; lymphocytes, 33 per cent.; large mononuclears, 5 per cent.; transitionals, 2 per cent.; eosinophils, 2 per cent.; basophils, 0.5 per cent. Inquiry showed that he had eaten heavily of cabbage and carrots and scarcely ever ate any other vegetables.

A diagnosis of carotinema was made and carrots were eliminated from his diet immediately. He was kept under observation for a period of six days during which a perceptible fading of the color occurred. About 20 c.c. of blood were drawn by venipuncture. A small amount of the blood was allowed to coagulate in a test tube, and the supernatant serum showed a bright golden yellow color (Fig. 1) which contrasted strongly with the straw color of normal serum (Fig. 2).

The remainder of the blood was oxalated and sent to Leroy S. Palmer, associate professor of agricultural biochemistry of the University of Minnesota, whose brilliant researches with carotin have been mentioned previously. His report reads:

> I have examined the sample of blood from the case of suspected carotinemia and beg to report as follows: There is no question regarding the presence of carotin in relatively large amounts in this blood, the serum color being due almost exclusively to this pigment. It is likely that traces of xanthophylls, the other class of carotinoids, are present also. This being the first sample of human blood I have had an opportunity to examine for carotinoid pigments, I took occasion to obtain an approximate estimation of the amount of pigment present. So far as I am aware, no measurement of carotinoids in human blood has heretofore been made, although a procedure for the determination of these pigments in other materials has been worked out. The result of the quantitative determination showed approximately 0.00057 gm. carotin per 100 c.c. of blood. This is, of course, a very small amount. However, carotin is a very intense pigment. Just how intense it is may be judged from the fact that butterfat from cows on fresh pasture grass contains on the average 0.025 gm. carotin per kilogram of fat. Assuming 5 per cent. blood in the human body, an individual weighing 75 kg. with 0.00057 gm. carotin per 100 c.c. of blood would have sufficient carotin in the entire blood to color about 1 kg. of "June" butterfat. I thought these figures might interest you.
>
> Permit me to say a word regarding the application of this examination to the case in question. It is only fair to point out that the finding of carotin in this blood is not necessairly a sure criterion of the carotinemia suspected. Carotin is unquestionably a normal constituent of human blood, the amount depending on the character of the diet. Carotinoids have already been reported in human blood from individuals presumably not suffering from carotinemia. I have found the pigments to be normal, although of varying constituence, in human milk fat. Human adipose tissue is notoriously yellow, as you know, due to the same pigments. The only sure index of the carotinemia suspected will be the disappearance of the pigment from the skin, at least so it seems to me, now that the source of the pigment has been removed. This will be slow, judging from my experience with animals and the cases reported by Hess with children. It might be of interest to examine the blood again.

After being on the carrot-free diet for one month the patient wrote: "The yellow is very nearly gone, the face and the rest of the body is clear. The palms of the hands show a trace of it yet. I am getting along fine." After two months he wrote: "The yellow color is very nearly gone but there is a trace of it left yet on the inside of my fingers."



A, orange yellow color of serum of patient, J. B. B, straw color of normal serum for comparison.

SUMMARY

We report a case of marked orange yellow skin pigmentation, most intense in the palms of the hands, without involvement of the sclerae, in an adult with moderately severe diabetes. Carrots had formed a heavy component of the previous diet. The blood serum showed a bright golden yellow color which was chemically demonstrated to be due to carotin. The urine showed no bile pigments, and withdrawal of the carrots from the diet caused a disappearance of the pigmentation.

FOCAL INFECTION AND ELECTIVE LOCALIZATION IN THE ETIOLOGY OF MYOSITIS *

EDWARD C. ROSENOW, M.D., AND WINIFRED ASHBY, PH.D.
ROCHESTER, MINN.

Although acute muscular rheumatism is generally conceded to be an infectious process, the myalgias and milder forms of myositis are not so definitely accepted as such. Myalgic pains are considered by Taylor [1] to be the result of faulty metabolism, by Gordon [2] of excessive katabolism from over-exertion of muscle cells, and by Profanter [3] as the peripheral expression of pain caused by defective function or injury of visceral organs. Except among English writers, such as Poynton,[4] Llewellyn and Jones,[5] Munroe and Almond,[6] and Stockman and Renton,[7] little credence is given to the idea of localized invasion of the muscles by bacteria other than in marked acute generalized myositis, and the condition described as dermatomyositis.

The demonstration by one of us (Rosenow [8]) eight years ago that streptococci from patients with rheumatic fever with muscular involvement, and streptococci from patients with acute nonrheumatic myositis,[9] tended to localize and produce nonsuppurative lesions in muscles of animals following intravenous injection supports strongly the infectious theory of myositis. The localization in muscles occurred with strains isolated from the focus of infection as well as those from the involved muscles.

There are many reasons for believing that chronic localized infections, such as in tonsils and teeth, play an important part in the production of pronounced and mild forms of myositis. Infections in these areas are commonly present in patients suffering from myositis. Localized myositis occurs often during certain epidemics of tonsillitis and other infections of the upper respiratory tract. Rheumatic fever in

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* From the Division of Experimental Bacteriology, The Mayo Foundation.

1. Taylor, J. M.: On Myalgic Pains, Lancet **2**:976, 1919.
2. Gordon, A.: Myalgia of an Isolated Portion of a Muscle, New York M. J., **107**:113, 1918.
3. Profanter, P.: Das Problem des Muskelrheumatismus, Ztschr. f. diätet. u. physik. Therap. **23**:121, 1919.
4. Poynton, F. J.: A Contribution to the Study of Acute Rheumatism, Brit. M. J., **1**:371, 1919.
5. Llewellyn, L. J., and Jones, A.B.: Fibrositis, New York, Rebman Co., (n.d.), pp. 693.
6. Munroe and Almond: Quoted by Llewellyn and Jones.
7. Stockman and Renton: Quoted by Llewellyn and Jones.
8. Rosenow, E. C.: The Etiology of Articular and Muscular Rheumatism J. A. M. A. **60**:1223 (April 19) 1913.
9. Rosenow, E. C.: Elective Localization of the Streptococcus From a Case of Pulpitis, Dental Neuritis and Myositis, J. Immunol. **1**:363, 1916.

which involvement of muscles is common is usually ushered in by an attack of tonsillitis. Beneficial effects from tonsillectomy were noted by Lillie and Lyons [10] in a study of 200 cases of myositis and arthritis, by Nordlund [11] in thirty cases, and by Kelly [12] in ten cases. The nature of the causal relationship, however, is generally considered to be other than the localization of bacteria in the muscles. Krebs [13] and Schmidt [14] put forward the idea that the symptoms in these conditions are due to injury of the nerves by toxins produced by the bacteria in a focus of infection.

The difficulties in studying the etiology of myositis, especially the milder forms, are many. Attacks of myositis are rarely fatal. Cultures from the blood prove negative. The lesions often occur in inaccessible parts. Removal of pieces of muscle for cultural and other studies is painful and objected to by many patients, and the isolation of bacteria free from contamination is difficult. We endeavored to obtain light on the nature of this puzzling problem by testing the effect in suitable animals of the intravenous injection of the bacteria from foci of infection, and excised pieces of muscles in cases of myositis and closely related conditions, and by noting clinically the effect on the course of the disease of the removal of the suspected foci of infection.

TECHNIC

The technic has been similar to that employed in other elective localization studies.[15] Whenever possible, material obtained from the depths of the suspected focus of infection, that is, material expressed from tonsils or aspirated from the depths of pus pockets of teeth showing pyorrhea, and the washings from ground-up tonsil tissue, was injected intravenously immediately. Cultures were made in tall columns (from 10 to 12 cm.) of ascites-glucose broth or glucose-brain broth (0.2 per cent.), and on blood-agar plates. To each tube of broth adjusted to hydrogen ion of 6.7 was added approximately 2 c.c. of calves' brain before autoclaving. The animals were injected intravenously with either the young primary broth culture or with the centrifuged organisms suspended in salt solution. From 2 to 5 c.c. of the broth culture itself, or the growth from 10 to 30 c.c. in salt solution were used routinely in average sized rabbits. The dose for guinea-pigs and dogs was in proportion. Smears and blood-agar plates were made of the material injected to determine the character of the organisms and their viability. Cultures made

10. Lillie, H. I., and Lyons, H. R.: Tonsillectomy in Myositis and Arthritis, J. A. M. A. **72**:1214 (April 26) 1919.

11. Nordlund, H.: Outcome after Tonsillectomy for Rheumatism, Hygiea, **81**:497, 1919; Abstr. J. A. M. A. **73**:568, 1919.

12. Kelly, T. H.: The Result of Animal Inoculations with Material Obtained From the Tonsils of Cases of Acute Rheumatic Fever, Ohio M. J. **14**:221, 1918.

13. Krebs, W.: Beitrag zur Frage der Myalgie und ihrer Behandlung, Ztschr. f. diätet. u. physik. Therap. **23**:288, 1919.

14. Schmidt, A.: Zur Pathologie und Therapie des Muskelrheumatismus (Myalgie), München. med. Wchnschr. **1**:593, 1916.

15. Rosenow, E. C.: Elective Localization of Streptococci, J. A. M. A. **65**:1687 (Nov. 13) 1915; Studies on Elective Localization. Focal Infection with Special Reference to Oral Sepsis, J. Dent. Res. **1**:205, 1919.

from plated material were not used routinely, for it has been shown that the bacteria having elective localizing power may be sensitive to oxygen, especially in the primary culture, and often fail to grow on blood-agar plates, and when not, aerobic cultivation has been found to destroy the peculiar pathogenicity which enables them to invade specific tissues. In shake cultures of excised pieces of muscle, in tall columns of agar the organisms may retain their viability and specific localizing power for a long time (Case 130).

Usually only one injection was given; in some instances two were given, and rarely three. The animals were often caged separately, observed for evidence of muscle symptoms, and handled with care to avoid injury. Those that died were examined as soon after death as possible. Those that survived were etherized, as a rule, in from forty-eight to seventy-two hours after the last injection and examined at once. In special instances they were allowed to live for from ten to fourteen days. Painstaking search for focal lesions in muscles and viscera was made in a strong light, and the incidence of lesions recorded.

The identity of the organism causing the lesions was determined by making routine cultures on blood agar and in tall columns of the broth of emulsion of the lesions in the muscles, of the blood (0.2 c.c.), and, in some instances, of emulsions of normal muscle, and viscera showing no lesions, and by microscopic examination of stained sections of the tissues. The organism isolated from the lesions in the muscles was frequently reinjected one or more times.

RESULTS

In this way we studied altogether twenty-eight cases of myositis, extending over a period of six years. The important clinical and experimental findings in these cases are summarized in Tables 1, 2 and 3. The age of the patients ranged from 6 to 65 years. All patients, except one, were 20 or more years of age. Sixteen were males and twelve were females. The condition found in the patients varied from mild acute transient, often recurring, attacks of localized myositis in persons who were well otherwise, to marked chronic generalized myositis which had incapacitated them for work for several years. Some of the patients had had attacks of other diseases, such as cholecystitis, but these conditions were not manifest at the time of our study. In six cases one or more other members of the family were similarly afflicted. There was a history of tonsillitis or sore throat in relation to attacks or exacerbations in ten cases. The tonsils were usually small and appeared to be quite normal on inspection, but by means of pressure pus was often expressed, especially from the pole, indicating probable absorption of bacteria and their products.

The tonsils alone were removed as the focus of infection suspected of having causal relationship in thirteen cases, teeth alone in four cases, and infected teeth and tonsils in eight cases. In three cases no foci were removed. In these cases the muscle trouble was preceded by tonsillitis in two and by nasopharyngitis in one.

The extirpated tonsils often showed one or more abscesses near the base. The infections of the teeth included pyorrhea (Case 601), infection of the pulp without apical rarefaction (Case 567), and apical

TABLE I.—Summary of Clinical and Experimental Findings in Cases of Uncomplicated Myositis

Case	Age, Sex	Clinical Findings	Foci Removed	Result	Bacteria Having Elective Localizing Power	Animal Experiments												
							Number Showing Lesions in											
						Animals Injected	Joint Fluid (turbid)	Periarticular Ligaments	Muscles	Nerves	Stomach and Duodenum	Kidneys	Lungs	Thyroid	Skin	Pericardium	Myocardium	Endocardium	Other Organs
130	21 F	Tonsillitis and puerperal fever two years before, followed by tender, painful nodes in the muscles of the neck and arms, and thyroid gland; excised nodes from neck showed calcifying myositis	None	.....	Slightly hemolyzing streptococcus from muscle	38	4	1	29	0	6	3	1	6	3	0	10	0	Gallbladder, suprarenals, appendix, pancreas, and liver in one each; intestines, thymus lymph gland, and eyes in two each
593	36 F	Acute pain in localized areas in muscles of back following tonsillitis; spontaneous recovery	None	.....	Undetermined	1	0	0	1	0	0	0	0	0	1	0	0	1	
594	6 M	Acute pain in muscles of shoulder and neck following nasopharyngitis; spontaneous recovery	None	.....	Undetermined	1	0	0	1	0	0	1	0	0	0	0	0	0	
602	26 M	Cervical myositis following tonsillitis.....	Tonsils	Unknown	Slightly hemolyzing streptococcus and staphylococcus from tonsils	4	1	0	4	0	1	2	0	0	0	0	3	0	Testicle in one
623	36 M	Myositis of right gluteus maximus of four years' duration; marked improvement since tonsillectomy one year before; tender tooth found and removed; calcifying pulpitis	Infected tooth	Complete recovery	Slightly hemolyzing streptococcus from calcifying dental pulp	2	1	0	2	0	0	0	0	0	0	0	0	0	
624	48 M	Chronic lumbar myositis for the last eight years following attacks of tonsillitis; septic tonsils; in bed for eight weeks with sciatica one year before	Tonsils	Complete recovery during last two years	Green-producing streptococcus from tonsils	2	0	0	2	0	0	0	0	0	0	0	0	0	
904	37 M	Marked chronic generalized myositis which began two years before with severe pain in muscles of back and legs after exposure to wet and cold; muscles and joints stiff and cold; eosinophilia of 4.7 per cent.; leukocytes 5,000; urine normal; tonsils infected	Tonsils	Gradual but complete recovery from myositis	Slightly hemolyzing streptococcus from tonsils	7	2	1	6	1	0	2	0	0	0	1	1	1	Brain in one; liver in two
909	26 F	Recurring attacks of tonsillitis, followed by cervical myositis; the attack studied came on ten days after acute follicular hemolytic streptococcic tonsillitis associated with fever and moderate leukocytosis; abscesses in extirpated tonsils	Tonsils	Recovery	Green-producing and indifferent streptococcus from tonsils	11	3	2	11	0	0	1	2	0	0	0	2	0	
912	30 F	Mild recurring attacks of localized myositis, and cholecystitis; two attacks of cholecystitis, one associated with pericarditis; one infected tooth, and infected tonsils	Tonsils and one infected tooth	Improvement	Slightly hemolyzing and green-producing streptococcus from tonsils and tooth	12	1	1	12	0	1	0	0	0	0	0	0	0	
1035	20 F	Mild recurring attacks of localized myositis, and probably cholecystitis; attacks usually followed tonsillitis; tonsils showed a number of abscesses	Tonsils	Improvement	Slightly hemolyzing streptococcus from tonsils	12	2	0	11	0	0	1	0	0	0	0	0	0	
		Total (10 cases).....	.....	.....	.....	90	14	5	79	1	8	10	3	6	4	1	16	2	

TABLE 2.—Summary of Clinical and Experimental Findings in Cases of Myositis and Arthritis

Case	Age, Sex	Clinical Findings	Foci Removed	Result	Bacteria Having Elective Localizing Power	Animal Experiments													
						Animals Injected	Number Showing Lesions in												
							Joint Fluid (turbid)	Periarticular Ligaments	Muscles	Nerves	Stomach and Duodenum	Kidneys	Lungs	Thyroid	Skin	Pericardium	Myocardium	Endocardium	Other Organs
164	40 M	Recurring localized myositis; subacromial bursitis, typical painful shoulder; many small abscesses in tonsils; infected devitalized teeth; painful bleeding hemorrhoids, with superficial ulceration	Tonsils and two infected teeth	Marked improvement	Slightly hemolyzing streptococcus from tonsils and teeth	8	2	1	5	0	3	0	0	2	0	0	1	2	Gallbladder in one
369	25 M	Chronic localized myositis, chiefly of muscle insertions around joints, and chronic arthritis	Tonsils, tonsil tag, and infected tooth	Improvement after tonsillectomy; complete recovery after removal of tonsillar tag and tooth	Green-producing streptococcus from tonsils, tooth, and joint fluid	8	4	2	4	0	3	1	0	0	2	3	3	2	
536	27 M	Muscular and articular rheumatism for three years; repeated attacks of tonsilitis	Tonsils	Decided improvement, but not complete recovery	Staphylococcus aureus from tonsils	4	2	1	4	0	1	2	0	0	0	0	3	1	Bladder in one
542	42 F	Repeated attacks of rheumatic fever, preceded by attacks of sore throat; chronic myositis and arthritis, chiefly of knees, elbows, shoulders, and back; hypertrophic rhinitis; follicular pharyngitis; large septic tonsils; two abscessed teeth	Tonsils and two abscessed teeth	Gradual improvement after tonsillectomy; rapid improvement after removal of abscessed teeth	Slightly hemolyzing streptococcus from tonsils and teeth	1	0	1	1	0	0	0	0	0	0	0	0	0	

543	65 M	Chronic myositis and periarthritis; septic tonsils	Tonsils	Marked improvement	Slightly hemolyzing streptococcus from tonsils	2	1	1	2	0	0	0	0	0	0	0	1	2	Gallbladder in one
552	51 M	Rheumatic fever when a child; repeated attacks of muscular and neuralgic pains; lumbago and sciatica for twelve years; repeated sore throat; pericarditis three years before; myositis and arthritis of right knee, but no sciatica at time of examination; abscesses in tonsils	Tonsils	Complete recovery soon after removal of tonsils five years before	Green-producing streptococcus from tonsils	2	1	0	2	0	1	0	0	0	0	0	0	1	
621	53 F	Chronic localized myositis, periarthritis and arthritis; tonsillectomy seven years before, with temporary improvement; infected tonsil tissue at base of tonsillectomy scar; dental abscesses	Tonsil tag, and two infected teeth	Decided improvement, but not complete recovery	Green-producing streptococcus from tonsils and infected teeth	4	3	3	2	0	1	0	0	0	0	0	1	0	Appendix, and hyperemia of pulps of teeth, one each
622	42 M	Chronic recurring localized myositis; subacromial bursitis, and low grade peripheral arthritis of fingers; tonsillitis; infected tonsillar tissue following incomplete tonsillectomy; dental infection	Tonsils and infected, devitalized teeth	Repeated improvement of systemic condition following removal of foci	Slightly hemolyzing and green producing streptococcus from tonsils and teeth	17	3	5	12	0	0	1	2	0	0	0	1	0	Bursae in nine; bladder in one
898	34 F	Chronic arthritis and myositis; marked exacerbation following quinsy three months before; tonsils small and ragged; apical infection of two devitalized teeth	Tonsils	Unknown	Green-producing streptococcus from tonsils	1	0	0	1	0	0	0	0	0	0	0	1	0	
913	37 F	Chronic localized myositis, periarthritis, and bursitis; submerged, small, septic tonsils	Tonsils	Complete recovery	Slightly hemolyzing streptococcus from tonsils	12	1	5	11	0	1	1	1	0	1	1	1	0	Bursae in four; lymph glands in two; spleen in one
922	64 F	Chronic myositis and arthritis, and paralysis agitans	One infected tooth	Temporary improvement of joint and muscle symptoms	Green-producing streptococcus from tooth	2	0	0	2	0	0	0	0	0	0	0	0	0	
		Total (11 cases).....	.....	.....	.....	61	17	19	46	0	10	5	3	2	3	4	12	8	

infections of artificially devitalized teeth (ten cases). Of the twenty-five patients from whom one or more suspected foci were removed, improvement or complete recovery, which was attributable to this treatment, occurred in twenty-two. In two cases (Cases 602 and 898)) the result is unknown, while in one case (Case 572) no improvement followed. The improvement in many instances occurred promptly, especially in the more acute cases. In the more chronic and more extensive infections the improvement was slow and sometimes did not become apparent until several months had elapsed. The good effects were not merely temporary; complete recovery has lasted in many of the cases for a number of years. In three cases partial recovery, noted after a previous tonsillectomy, became complete, or additional improvement occurred following the removal of the dental infections (Cases 567, 623, and 4132). In two patients complete restoration to health followed removal of tonsillar tissue which remained after a defective tonsillectomy, and the removal of one or more infected teeth (Cases 369 and 622).

In all cases but one, a patient with spasmodic torticollis (Case 572), in whom no improvement followed tonsillectomy, elective localization of bacteria from foci or from excised muscle occurred following their intravenous injection into animals. Of the twenty-seven positive cases, the streptococcus was found to have elective affinity for the muscles in twenty-four cases, the streptococcus and staphylococcus in two cases, and the staphylococcus alone in one case.

The streptococcus produced two types of colonies on blood-agar plates, one, a small colony surrounded by a narrow and usually a somewhat hazy zone of hemolysis, the other, a slightly larger but dry colony surrounded by a greenish zone. The muscle lesions in animals were caused by the slightly hemolyzing streptococcus in ten cases and by the green-producing type in ten cases; in four cases both varieties were present, and in two cases the hemolyzing streptococcus and staphylococcus (Case 602) were responsible. In one case the *Staphylococcus aureus* alone (Case 536) was the causative factor.

In most instances, the details of the patients' symptoms were not known to us when the results of the experiments were recorded. In reviewing the clinical histories, we found that the cases fell into three quite distinct groups. The cases in Group 1 (summarized in Table 1), comprise the cases of acute and chronic myositis without other demonstrable lesions at the time of study. In Group 2 (Table 2) are the cases that presented predominating symptoms of myositis, but in which periarthritis and arthritis were also present. In Group 3 (Table 3) are the cases in which the findings were chiefly those of myositis, but in which symptoms of mild neuritis or perineuritis were

TABLE 3.—Summary of Clinical and Experimental Findings in Cases of Myositis and Neuritis

Case	Age, Sex	Clinical Findings	Foci Removed	Result	Bacteria Having Elective Localizing Power	Animal Experiments													
						Animals Injected	Number Showing Lesions in												
							Joint Fluid (turbid)	Periarticular Ligaments	Muscles	Nerves	Stomach and Duodenum	Kidneys	Lungs	Thyroid	Skin	Pericardium	Myocardium	Endocardium	Other Organs
567	28 F	Neurasthenia; migraine; recurring acute myositis of muscles of neck, associated with dental neuritis or facial neuralgia; cervical lymphadenitis; colloid goiter; three infected dental pulps; previous tonsillectomy with improvement	Infected teeth	Marked improvement, but not complete recovery	Slightly hemolyzing streptococcus from teeth, and excised muscle	23	0	1	15	12	3	3	1	0	0	0	0	0	Teeth in eleven; gallbladder in two; suprarenals, spleen, and lymph glands, one each
571	22 F	Neurasthenia; migraine; pains in muscles of chest, especially the left side, and in the back; numbness of right arm, especially when fatigued and nervous; hacking cough, and nausea; infected tonsils; cloudy right antrum	Tonsils	Marked improvement	Green-producing streptococcus from tonsils	5	2	1	5	2	1	1	1	0	0	0	1	1	
572	43 M	Spasmodic torticollis for four months....	Tonsils	No change in condition	None	2	0	0	0	0	0	0	0	0	1	0	0	0	
601	43 M	Repeated attacks of muscular rheumatism for years; at time of examination impaired conduction and heart block; marked pyorrhea; apical infection of three teeth	Three teeth; prophylactic treatment of pyorrhea	Marked improvement lasting two years	Slightly hemolyzing streptococcus from teeth	13	0	6	7	2	2	0	5	0	0	3	5	6	Spinal cord, aorta, intestines, two each; gallbladder, thymus, epicardial fat one each
923	44 M	Neurasthenia; lack of endurance; muscular pains, especially of the chest and shoulders; numbness in arms at times; apical infection around three teeth; septic tonsils	Tonsils, and three infected teeth	Gradual improvement	Green-producing streptococcus from tonsils and teeth	4	2	0	3	0	1	0	0	0	0	0	0	0	
2291	49 M	Neurasthenia; lack of endurance; muscular and nerve pains in lumbar region, and thighs; septic tonsils	Tonsils	Gradual improvement	Staphylococcus aureus from tonsils	2	0	0	2	1	0	0	1	0	0	0	0	0	
4132	52 M	Acute localized myositis and dental neuritis; previous tonsillectomy with improvement	Three infected teeth	Prompt improvement	Green-producing streptococcus from teeth	2	0	0	2	1	0	0	0	0	0	0	0	0	Teeth in two
		Total (7 cases).....	.....	.....	.....	51	4	8	34	18	8	4	8	0	1	3	6	7	

also present. The incidence of lesions in each of the three groups is given in Tables 1, 2 and 3. It will be noted that in Group 1, the pure myositis group, seventy-nine (88 per cent.) of ninety animals injected had lesions in muscles; fourteen (16 per cent) animals had turbid joint fluid, and only 1 per cent. had lesions in nerves. In Group 2, that of myositis and arthritis, lesions of muscles occurred in forty-eight (79 per cent.) of the sixty-one animals injected; seventeen (28 per cent.) had turbid joint fluid, and none developed lesions in nerves. In Group 3, that of myositis and neuritis, lesions in muscles developed in thirty-four (67 per cent.) of fifty-one animals; only four (8 per cent.) had turbid joint fluid, and eighteen (35 per cent.) had lesions in nerves or nerve sheaths. The incidence of lesions in the periarticular ligaments in the three groups was 6 per cent., 31 per cent. and 16 per cent., respectively. This parallels quite closely the patients' symptoms, not only with regard to the chief lesions in the muscles, but also in the minor lesions, such as those in the joints in the arthritis group and in the nerves in the neuritis group.

TABLE 4.—Elective Localizing Power of Streptococci from Myositis as Compared with Streptococci from Other Sources

Source of Streptococci	Strains	Animals	Percentage of Animals Showing Lesions in												
			Muscles	Joints and Ligaments	Nerves	Stomach and Duodenum	Gallbladder	Appendix	Kidneys	Lungs	Skin	Pericardium	Myocardium	Endocardium	Central Nervous System
Myositis	28	202	**80**	33	9	13	3	1	9	7	4	4	17	8	?
Ulcer of stomach	37	168	4	12	0	**68**	21	1	3	0	0	2	3	10	?
Cholecystitis	12	41	7	17	0	29	**80**	0	5	5	2	0	2	10	?
Appendicitis	17	71	12	29	0	11	1	**70**	0	0	0	0	9	21	?
Acute poliomyelitis	22	123	16	15	4	13	2	2	2	11	0	5	7	7	**46**
Miscellaneous	71	212	12	9	4	9	1	1	9	7	4	0	4	12	?

In Table 4 is summarized the total incidence of lesions in the three groups of cases of myositis, and, for purposes of comparison, the incidence of localization in similar experiments in ulcer of the stomach, cholecystitis, appendicitis, poliomyelitis and of streptococci from similar sources from persons having miscellaneous diseases, and from normal persons. Among the diseases studied in the miscellaneous group were acute myelogenous leukemia, urticaria, ulcerative colitis, bronchitis, elephantiasis, thyroiditis, diabetes, purpura, jaundice, epididymitis, acute pyorrhea, dermatitis and angina pectoris. The incidence is given in percentages, so that the figures are directly comparable. Thus, of 202 animals injected with twenty-eight myositis strains, 161 (80 per cent.) developed lesions in muscles, and sixty-seven (33 per cent.) developed arthritis, manifested by turbidity of joint fluid or by hemor-

rhages in the periarticular ligaments. The total incidence of lesions in the muscles in each of the other diseases studied, and in the miscellaneous group, is much lower, varying from 4 per cent. in ulcer to 16 per cent. in acute poliomyelitis. The high figure in each of the other diseases corresponds to the disease the patients were suffering from at the time of the experiments. Thus, in ulcer, 68 per cent. of the 168 animals developed lesions in the stomach or duodenum; in cholecystitis, 80 per cent. of forty-one animals developed cholecystitis; in appendicitis, 70 per cent. of seventy-one rabbits developed lesions in the appendix; in acute poliomyelitis, 46 per cent. of 123 animals developed lesions in the central nervous system. This is in sharp contrast to the results obtained following the injection of 212 animals with seventy-one strains from miscellaneous sources. In these, as shown in the last line in Table 4, no particular organ was attacked. The incidence of lesions of 12 per cent. in voluntary muscles is about that shown in Henrici's [16] tables following injection of plated streptococci from miscellaneous sources. The myocardium in the myositis group had lesions, often marked, in thirty-four (17 per cent.) of the animals, which, as might be expected, is a considerably higher incidence than occurred following the injection of other strains; in the case of ulcer it was 3 per cent., in cholecystitis, 2 per cent., in appendicitis, 9 per cent., in poliomyelitis, 7 per cent. and following miscellaneous strains, 4 per cent.

These percentages, striking as they are, do not express adequately the differences observed at necropsy, because one lesion found in an animal injected with a nonmyositis culture counts for as much in the table as many lesions in an animal following the injection of the myositis strains.

In two cases in which there was a history of peridental symptoms (Cases 567 and 913), lesions of pulps of teeth or adjacent periosteum occurred in 50 per cent. of thirty-five animals injected. The remarkable tendency of these strains to localize electively (Table 4) was paralleled in individual instances in which localization in animals was not only in muscles, but chiefly in the same group of muscles, as was noted in the patient. Thus, in myositis of the gluteus muscles (Case 621) the localization was chiefly in this group. In two cases of cervical myositis (Cases 567 and 909), the chief localization occurred in the muscles of the neck. In two cases of intercostal myositis, the localization was almost limited to the intercostal muscles, and in one case this was true in two sets of experiments (Fig. 2, III and IV). In the case of lumbar myositis (Case 624) the lesions in the rabbits were chiefly in the lumbar muscles (Fig. 2, II), a finding which supports the idea that infection may play a part in the etiology of chronic lumbago. In Case 913, in which the chief complaint was referable to the periarticular

16. Henrici, A. T.: The Specificity of Streptococci, J. Infec. Dis. **19**:572, 1916.

structures of the right knee, the striking localization occurred in animals around the knee joints (Fig. 10, IV). The lesions in the muscles of the animals injected with the bacteria from the myositis-neuritis group were frequently situated around nerves (Fig. 2, I, III and Fig. 3, I), and the lesions in nerves independent of muscle lesions sometimes corresponded to those involved in the patient. This was particularly true in Case 567 (Table 3), in which there were recurring attacks of facial neuralgia due to pulpitis and dental neuritis, and cervical myositis. The affinity of the slightly hemolyzing streptococcus isolated from the foul dental pulp and the excised muscles of the neck for dental pulps and dental nerves, and muscles of the neck, was so marked that lesions in these structures developed even after intraperitoneal injection. Besides lesions in muscles, arhythmia of the heart, with lesions in the bundle of His and in the myocardium, developed in animals following the intravenous injection of streptococcus from the pyorrheal pockets in Case 601, the patient who had had repeated attacks of muscular rheumatism and who was suffering from attacks of syncope due to a damaged His bundle and impaired myocardial conduction. Localization in muscles occurred in experiments performed during repeated attacks in some patients; for example, three times each in Cases 622 and 912, and five times in Case 1,035. In one patient (Case 912) localization in muscles of animals occurred as the chief lesion during each of three widely separated attacks of acute localized myositis. Lesions in the gallbladder, myocardium and pericardium, without muscle lesions, were produced during an attack of cholecystitis and pericarditis, with dilatation of the heart, and lesions in the stomach or duodenum developed in the animals injected during two attacks of indigestion. There were few or no lesions in the animals injected in the same way while the patient was free from symptoms on three widely separated occasions.

The strains from generalized myositis tended to produce numerous small lesions in many muscles, sometimes thousands of lesions, while those from localized myositis tended to produce a few localized areas of muscle involvement.

The objection which has been raised regarding dosage has been met not only by injecting smaller doses of cultures, but by directly injecting salt solution suspensions of the small number of bacteria in the pus expressed from tonsils. Indeed, some of the most specific localizations occurred in these experiments. It was thought worth while, therefore, to summarize the results obtained by injecting the small doses of the bacteria which had grown in the focus itself.

Lesions in muscles developed in all of fourteen rabbits injected with the pus from five patients in the uncomplicated myositis group. Two rabbits developed arthritis, and one rabbit had a hemorrhage of the

stomach in addition. Of the fifteen rabbits injected with the tonsil pus from nine patients in the myositis and arthritis group, fourteen developed lesions in the muscles, ten in the joints, two in the stomach, two in the myocardium, and one in the endocardium. Of the six rabbits injected with the tonsil pus from five patients in the myositis and neuritis group, five developed lesions in the skeletal muscles, one in the stomach, two in the myocardium, and three in the nerves. Thus, of the total of thirty-five rabbits injected, thirty-three (94 per cent.) had lesions in muscles. The results obtained following injection of the tonsil pus from twelve persons who were well at the time or who

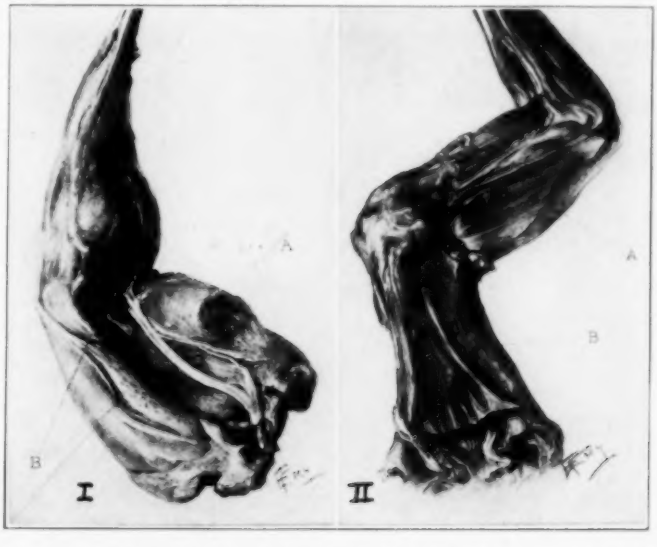

Fig. 1.—(I) Numerous small hemorrhagic lesions in the muscles of the hind leg of a rabbit forty-eight hours after injection of the primary culture of the streptococcus from the tonsils in a case of acute cervical and intercostal myositis (Case 909). (II) Numerous whitish necrotic lesions of the muscles of the fore leg and shoulder of a rabbit eight days after injection with the streptococcus from the tonsils after one animal passage in a case of marked but chronic myositis (Case 904).

had some ailment not attributable to infection were in sharp contrast. Of the twenty-four rabbits injected, only three (12.5 per cent.) had lesions in muscles, and one rabbit had hemorrhages of the stomach.

The virulency of the bacteria injected was of a low order. Of a total of 202 animals injected (168 rabbits, seventeen dogs, nine guinea-pigs, seven mice, and one monkey), only forty-six died; 156 were anesthetized for examination. The results of cultures of the blood

made at necropsy were recorded in 144 instances. No growth was obtained in eighty-four; sixty cultures were positive. Streptococcus was found in forty-five, staphylococcus in nine, colon bacillus in eight, and a gram-staining bacillus in five. In most instances, the cultures were pure, although mixtures of streptococcus and staphylococcus were found occasionally. Cultures of the blood of the animals that were sacrificed remained sterile in 62 per cent. and showed growth in 38 per cent. The blood of the animals that died showed growth in 54 per cent. and remained sterile in 46 per cent. The number of colonies of streptococci on blood-agar plates in the positive cultures was relatively small. Often no growth occurred on aerobic plates when the deep broth cultures were positive. This was true even though the amount of blood inoculated was comparable in the two mediums. The oxygen sensitiveness was found also in the strains of streptococci isolated from the lesions in the muscles. Control culture of blood, and of various tissues without lesions, including normal muscles, were often sterile when cultures from the lesions in the muscles revealed streptococci. But even the lesions resulting from a single injection tended to become sterile so that cultures had to be made soon after injection, preferably in from twelve to forty-eight hours, in order to demonstrate the presence of living bacteria.

REPORT ON CLINICAL AND EXPERIMENTAL OBSERVATIONS

DETAILS OF THE MORE IMPORTANT CASES IN THE UNCOMPLICATED MYOSITIS GROUP (TABLE 1)

Case 1.—Mrs. O. E. R. [Case 130], aged 21, a farmer's wife, had had an attack of tonsillitis and puerperal septicemia two years before examination, and about the same time had noticed lumps in her neck. A few months before she came to the clinic the lumps had grown larger and more painful.

Examination revealed small, hard nodules in the right lobe of the thyroid, a chain of smaller lumps running down under the clavicle, lumps in the right axilla, and calcifying nodules along the biceps of the left arm. There was spasm of the left sternocleidomastoid. Operation was performed, and the calcifying masses which were found lying in the sternocleidomastoid muscle and extending out on each side of the muscles were removed from the right cervical region. The pathologist reported myositis ossificans.

A portion of the excised muscle was cultured Aug. 21, 1914. The glucose-agar-shake culture yielded twenty-four small white colonies of a pleomorphic streptococcus, which produced slight and hazy hemolysis on a blood-agar plate. This tube was layered with liquid petrolatum and preserved in the dark at room temperature for subsequent studies.

Subcultures in tall columns of glucose broth from single colonies in the shake culture were injected intravenously into animals on different dates. The first series of animals injected (September 14-18) consisted of ten rabbits, five dogs and three guinea-pigs. Eight rabbits and two dogs had localization in muscles. The primary culture of the streptococcus from the muscle of one rabbit and one dog was injected into seven rabbits and one dog. Five of the rabbits developed lesions of muscles; the dog developed acute hemorrhagic pancreatitis.

The primary culture of the streptococcus from two of the rabbits was reinjected into two rabbits (third animal passage). One had slight muscle lesions; the other died of overwhelming infection without focal lesions.

April, 1915, the second series of animals, consisting of three rabbits and two dogs, was injected with the subculture from a single colony in the agar tube. Two of the rabbits developed lesions in the skeletal muscles, myocardium and joints, and one had no lesions. Both dogs had hemorrhagic lesions of the flat muscles around the shoulders and thorax.

March 8, 1916, a rabbit and a monkey were injected with the broth culture from the growth in the original agar culture. Both developed hemorrhagic lesions in muscles and the monkey also had hemorrhages in the myocardium.

At this time, the streptococci from 300 c.c. glucose broth were dried and placed in a desiccator over calcium chlorid and kept from light. After the subculture was dried, it revealed a pure growth of the streptococcus. The one rabbit injected with a suspension in water developed a few small areas of hemorrhage, and infiltration of the flat muscles and fascia of the lateral aspect of the abdomen. One of these areas surrounded a small subcutaneous nerve. The

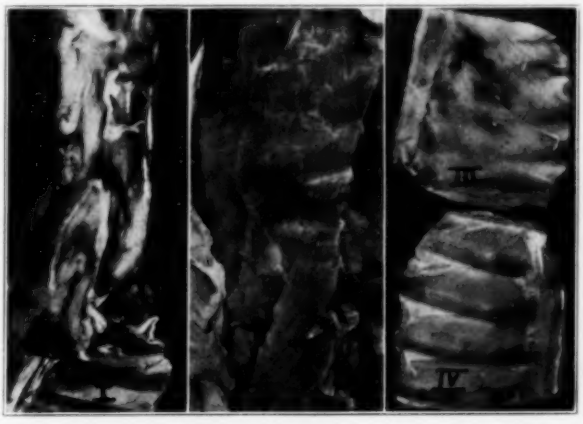

Fig. 2.—(I) Hemorrhagic lesions in the muscles of the neck surrounding the exit of the fifth cervical nerve of the rabbit injected with the tonsil pus in Case 909. (II) Localized area of hemorrhage, edema, and infiltration of the muscles of the lumbar region in a rabbit injected with the streptococcus from the tonsils in a case of lumbar myositis (Case 624). (III) Hemorrhagic lesions in the external aspect of the muscles of the upper thorax, especially around the exit of the small nerve trunks, in a rabbit injected with the streptococcus isolated from the mucopurulent discharge from the nose of a man at the time of an attack of intercostal myositis. (IV) Hemorrhagic lesions in the internal intercostal muscles of the thorax in the rabbit referred to in Figure 2 (III).

dried streptococci were kept until November, 1917, when it was found that cultures were negative. The effect of the dead organism suspended in salt solution was then tested by injecting two rabbits intravenously. Both developed hemorrhages in the flat muscles around the chest and shoulders and in the tendinous end of the muscles of the extremities.

Of the thirty-eight animals injected in this case (twenty-six rabbits, eight dogs, three guinea-pigs and one monkey), twenty-nine (76 per cent) had lesions in the skeletal muscles. Ten had myocarditis. The incidence of lesions

in other organs was low (Table 1). The fact that this organism lived and held its specific infecting power for so long (nineteen months) in the original shake culture, and the power of the dried dead streptococcal bodies to produce muscle lesions three years and four months after isolation, is nothing less than remarkable.

Case 2.—Mr. C. G. S. [Case 624], aged 48, eight years before examination had had a severe attack of tonsillitis, followed by a "dull aching pain," soreness and stiffness in the muscles of the back, which had been constant. A year previous to examination he was in bed for eight weeks with sciatica and lumbago. The patient had two sisters who were troubled with rheumatism.

The teeth were vital and free from pyorrhea. The roentgenogram did not reveal apical abscesses, and the roentgenograms of the lumbar spine were negative. The clinical diagnosis was chronic lumbar myositis and chronic tonsillitis. The tonsils were large and contained pus and caseous material. Tonsillectomy was performed. The emulsion of the extirpated tonsils and the primary culture were injected, respectively, into one rabbit each. Hemorrhagic lesions in muscles, most numerous in the hip and leg muscles of the lumbar region (Fig. 2, II), developed in both. There were no lesions elsewhere. One year and six months later, the patient reported that there was marked improvement and that shortly after the tonsils were removed the pain in the back almost disappeared. Four years later he reported that except for slight distress at times he was free from his former pain, soreness and stiffness in the muscles of the back, and was feeling well.

Case 3.—Mr. S. K. [Case 904], aged 34, an ice deliverer, had a severe exposure in the ice house, November, 1916, which was followed by intense pain in the back, extending to the legs; the attack was not preceded by any chill or fever. January, 1917, the patient had another attack with pain in the neck, back, arms and legs, again not preceded by chills or fever. He was taken to a hospital, and with daily application of hot packs for three weeks there was some improvement in his condition.

On examination May 9, 1917, all his muscles and joints were stiff and very painful, the pain being most severe in the muscles of the shoulders and arms. There was an eosinophilia of 4.7 per cent. The examination for trichina and the examination of the viscera were negative.

Pus was expressed from the depths of the tonsils and cultured in ascites-glucose broth. Plating showed an almost pure culture of distinctly hemolyzing streptococcus. The growth in glucose-brain broth was centrifuged and suspended in salt solution, and the growths from 7.5, 15 and 30 c.c., respectively, was injected intravenously into three young rabbits. After forty-eight hours, the rabbit which had received the growth from 30 c.c. died and a necropsy was performed while the body was still warm. The rabbit which had received the growth from 15 c.c. was sensitive to the touch. It had a convulsion, from which it recovered, and was then sacrificed. The rabbit which had received the growth from 7.5 c.c. appeared well and was sacrificed. On postmortem examination all three of these rabbits had small white linear lesions in the muscles, and in each rabbit these lesions were definitely more numerous in the muscles of the upper arm and shoulder than in other parts; their number was roughly in proportion to the number of bacteria injected. The rabbit injected with the smallest does had lesions of the skeletal muscles only, and gave a sterile blood culture. All organs in the rabbit injected with the 15 c.c dose were normal grossly, although microscopically the liver showed areas of slight infiltration. Cultures of the blood yielded streptococci. The muscle lesions consisted of dense leukocytic infiltration. The rabbit which received the 30 c.c. dose had lesions in the heart muscle and in the nerve sheaths, in addition to the numerous linear lesions in the skeletal muscles. There were no macroscopic lesions in the heart valves, stomach or kidneys, and the knee joint fluid

was normal. Cultures of the affected portion of the muscles in ascites-glucose broth in twenty-four hours yielded an apparently pure culture of streptococcus which, when plated on blood-agar, developed a fine, green-producing type of colony in large numbers, and a few staphylococcus colonies.

Two rabbits were injected with broth culture from the muscle. One, which had received 3 c.c., died at the end of forty-eight hours and a necropsy was performed before rigor mortis set in. A number of lesions were found in the muscles, most numerous in the triceps, deltoid and the muscles of the shoulder blade; areas of hemorrhagic infiltration were present in the periosteum and in the upper epiphysis of the right humerus, and a large number of embolic areas were noted in the kidneys, both in the cortex and in the medulla. Microscopic examination of the kidneys and muscles revealed sharply defined areas of dense leukocytic infiltration, with destruction of adjacent tissue. The second rabbit was injected with 3 c.c. of the same broth culture every second day for six days. On the eighth day it was sacrificed. The first day after the injection it was inactive, but apparently had no pain. On the second day, it was

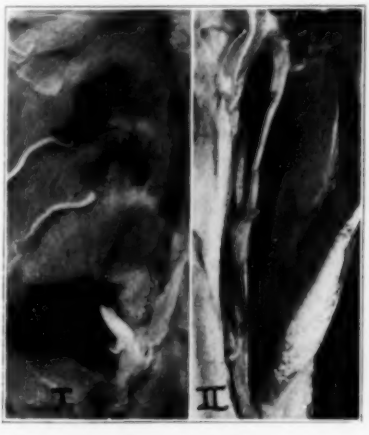

Fig. 3.—(I) Hemorrhagic lesions in the muscles, surrounding the nerve trunks, of the under surface of the scapula and thorax of a rabbit injected with the streptococcus from infected teeth of a man having myalgia, especially of the chest and shoulder muscles (Case 923). (II) Hemorrhagic lesions in the internal aspect of the muscles of the hind leg along the tendinous partition and adjacent nerve trunk in a rabbit injected with the streptococcus from the tonsils of a neurasthenic young woman at the time of localized myalgic pains of the thorax, and numbness of the right arm (Case 571).

better, but seemed sensitive. The next day its movements were slow and it showed a decided disinclination to walk. This tendency increased, until on the sixth day it refused to move unless shoved. On postmortem examination many white lesions were found, generously distributed through the trunk muscles, and especially numerous in the adductor group of the thigh, shoulder and deltoid. Culture of the knee joint fluid yielded streptococci. The blood and kidneys were sterile. Microscopic examination showed lesions in the muscles, kidneys and liver, consisting of areas of young scar tissue containing endothelial cells, large and small lymphocytes and fibroblasts, but no leukocytes. There were areas showing moderate calcification.

On the basis of the above findings the patient's tonsils were removed. An emulsion was made of them and 2 c.c. were injected into a rabbit. This rabbit developed arthritis of one knee and lesions in the muscles and the nerve sheaths.

Agglutination tests were made with the patient's serum against the culture of the patient's tonsils, the streptococcus growth from one of the rabbit muscle lesions, and the staphylococcus growth from the muscle lesions of the patient and one of the rabbits, but only negative results were obtained.

Marked lesions of the skeletal muscles were produced in four rabbits injected with the streptococcus from the tonsils of this patient. The animals receiving the larger doses had a few lesions in other tissues (Table 1), while those injected with minimal doses had lesions only in the skeletal muscles. The lesions in the muscles were more numerous in the shoulders and arms than elsewhere. Slightly hemolyzing streptococci were recovered from the muscle lesions in one of the injected rabbits, and produced marked lesions in the muscles of two rabbits into which the culture was injected.

Three weeks after the removal of the tonsils, the patient was decidedly better and was able to walk with ease. After two months, he wrote that he was still sore in the shoulders, but that otherwise he was well and free from pain, although there was still some weakness in the legs. Three months after the operation, he complained of not being so well and was sent a vaccine, which consisted of the streptococcus recovered from the tonsils and from muscle lesions in the rabbits. Three years and nine months later, he reported that he had been free from rheumatic trouble, but that he continued to be nervous and very weak. In view of the destructive lesions which the streptococcus from this patient produced in the muscles of rabbits, the persistent weakness is believed to be due to actual destruction of muscle fibers and the formation of connective tissue.

Case 4.—Mrs. P. L. [Case 909], aged 26, had an acute attack of follicular tonsillitis caused by the hemolytic streptococcus. Ten days later, the fever returned, accompanied by malaise, severe aching and general pain, at first associated with stiffness, and later with sharp pain and tenderness in the muscles of the left side of the neck, and a painful, tender area in the intercostal muscles above the left costal margin. The patient recovered promptly from this attack, but remained nervous and had mild attacks of pain in the muscles until seven months later, February, 1918, when her tonsils were removed. On dissection, a number of encapsulated abscesses were found in the tonsils. The patient has had better health than before and has been quite free from myositis.

The results of the animal experiments in this case were most interesting. The one rabbit injected with a small amount of pus expressed from the tonsil at the height of the attack of myositis was sensitive to touch and disinclined to move for two days. It was then chloroformed and numerous small hemorrhagic lesions in the muscles of the left side of the thorax, the inner aspect of the right thigh, and the lower end of the muscles of the right leg were found. The rabbit injected with the primary culture of the pus in ascites-glucose broth was extremely muscle-sore and weak when anesthetized forty-eight hours later. It had numerous small hemorrhagic lesions in the muscles of the extremities (Fig. 1, I), and large edematous hemorrhagic lesions in the deeper layers of the muscles of the neck. A few small lesions were found in the kidneys, papillary muscles of the heart, and cordi tendinae. The blood of both rabbits was sterile. The blood-agar plate of the pus from the tonsil showed chiefly green-producing and indifferent streptococci, and a few colonies of hemolytic streptococci. One rabbit was injected with the broth culture in glucose broth from single colonies of each of the three types of streptococci. All rabbits remained well and all were free from lesions of muscles. The rabbit injected with the hemolytic streptococcus developed suppurative arthritis.

Stab cultures of the pus from the tonsil were made in tall tubes of ascites-tissue agar. This was incubated for nine days and then preserved in the dark for subcultures for further animal experiments.

The twenty-four hour cultures in tall columns of ascites-glucose broth from the bottom of the agar stab were injected (from nine days to three weeks later) into five rabbits. All had lesions of the muscles, but the lesions were fewer in number and less marked than those following injection of the primary culture. The one rabbit injected with the growth after an additional transfer in ascites-glucose broth had lesions in the tendinous portion of the muscles around the legs and shoulders. The blood-agar plates made from the broth cultures in all of these animals contained green-producing and indifferent colonies of streptococci, but no hemolytic streptococci.

Seven months later, when the patient was free from myositis, two rabbits were injected with the primary culture in ascites-glucose broth of the pus expressed from the tonsils. Both remained well and both were found free from lesions at necropsy forty-eight hours after the injections.

Case 5.—A young woman [Case 912], a laboratory technician, has been under observation for a number of years. She is a hard, persistent worker, and her general health has been good, but she has had recurring attacks of

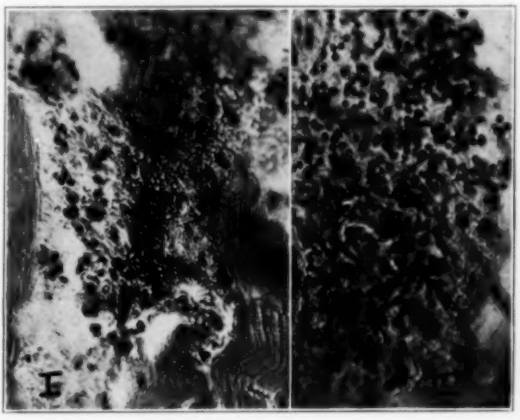

Fig. 4.—(I) Hemorrhage and leukocytic infiltration within and between the muscle fibers in the excised muscle in a case of acute rheumatic myositis in man. Hematoxylin and eosin (X 200). (II) Marked leukocytic infiltration in an excised section of muscle from the forearm of a patient with acute localized myositis. Hematoxylin and eosin (X 200).

mild localized myositis, several attacks of indigestion resembling ulcer or cholecystitis, and one severe attack of cholecystitis and pericarditis and myocarditis. In the winter of 1911, she developed severe pain in the intercostal muscles of the right lower chest, which persisted for some weeks and then gradually disappeared. No experiments were performed during this attack.

May, 1917, while the patient was well, four rabbits were injected as controls for some experiments in Case 913. Two rabbits were injected with a salt suspension of the pus expressed from the tonsils and two with the primary culture in tall columns in glucose broth. All the animals were well when chloroformed forty-eight hours later. The first two rabbits injected and one of the others, the one receiving the small dose, had no lesions. The rabbit receiving the large dose had a few small hemorrhages in the right biceps and in the intercostal muscles on the left side, and a few around the left knee joint.

Nov. 27, 1917, the patient developed pain in the muscles of the left arm and the right side of the abdomen. This came on without sore throat or ton-

sillitis, lasted for a few days, and then disappeared. On the first day of the attack, one rabbit was injected with an emulsion in salt solution of pus expressed from the tonsils. It was muscle sore when anesthetized the next day. Examination revealed a moderate number of small hemorrhages in the intercostal muscles on the right side, and the muscles of the scapula on the left side, and a large diffuse area of hemorrhage and edema in the subcapsular muscles on the right side.

Two weeks later, December 11, the patient developed, from no apparent cause, a sharp attack of indigestion. There was a feeling of fulness, distress and pain in the epigastrium for several days. Symptoms of hyperacidity developed from three to four hours after meals, which lasted for several weeks and then disappeared. At the height of this attack one rabbit was injected with pus expressed from the tonsils, and one with the primary culture in glucose broth. Both animals seemed to be well when chloroformed forty-eight hours after injection. The first rabbit had a hemorrhage in the pyloric end of the stomach and a few small hemorrhages in the intercostal muscles on the right side. The second rabbit had an ulcer in the pyloric end of the stomach, and whitish lesions in the gallbladder.

Feb. 23, 1918, while the patient was feeling perfectly well, one rabbit and one dog were injected with the primary culture from the tonsils for controls in Case 1,035. Both animals were well when chloroformed forty-eight hours later, and had no lesions.

February, 1919, the patient had an attack of sore throat followed by myositis involving several shoulder and intercostal muscles. At the height of the attack, a suspension of pus from the tonsils and the primary culture in broth were injected into one rabbit each. The rabbit injected with the first culture had scattered, large and small hemorrhages in the muscles throughout the body; the one injected with the second culture had a group of hemorrhages in the muscles of the thigh on the left side.

August, 1919, the patient became acutely ill with pain in the region of the heart, and some days later with pain in the region of the gallbladder. She had fever and leukocytosis. Pericardial friction rubs, dilatation of the pericardial sac and myocardium, and acute tenderness in the region of the gallbladder developed. She was in bed for four weeks and then gradually recovered completely.

Two weeks after the onset of this illness two rabbits were injected with the primary culture from the tonsil. Both animals appeared to be well four days later when they were chloroformed and examined. Pericarditis, evidently secondary to focal necrotic lesions in the myocardium, was found in both, and hemorrhage of the heart valve was found in one rabbit. There were no lesions of the stomach or gallbladder.

When the patient's convalescence was well advanced, a roentgen-ray examination was made of her mouth. The devitalized, crowned second right upper molar showed slight rarefaction at the apices of the roots. This tooth was extracted and the primary culture in glucose-brain broth from the apical end was injected intravenously into two half-grown rabbits. Both animals were apparently well when anesthetized two days later. Both had embolic whitish areas in the gallbladder, surrounded by hyperemic or hemorrhagic zones, and edematous and hemorrhagic pulps of teeth. One rabbit had, in addition, a few whitish hemorrhagic areas in the left ventricle. There were no lesions in the stomach or muscles.

After the extraction of the tooth, the only one showing pathology, and the removal of the tonsils, Sept. 30, 1919, on the basis of the experimental findings, the patient was free from stomach and gallbladder attacks, as well as from myositis, until Feb. 4, 1921, when she developed soreness in the back part of the throat and symptoms of acute tonsillitis. On examination, a rather large mass of red, tender tonsillar tissue, containing a decided crypt, was found at the base of each tonsillectomy scar. Following this attack, she devel-

oped marked tenderness and swelling of the cervical glands immediately adjacent to the tonsillar area, a tender spot in the left deltoid, soreness in several places in the muscles of the back, and a very tender and painful area in the muscles of the anterior outer and lower third of the left thigh. The tenderness in the muscles disappeared in four or five days and recovery was complete.

Three days after the onset of pain in the muscles of the left thigh, a small amount of pus was expressed from the hypertrophied tonsillar tissue on both sides. The cultures on blood agar directly, as well as from the primary culture in glucose-brain broth, contained large numbers of hemolyzing streptococci. Two rabbits were injected with the suspension of the pus, one from the right, the other from the left tonsil. Both developed necrotic and hemorrhagic lesions of muscles, but no lesions elsewhere. The primary culture in glucose-brain broth from each tonsil was injected into two rabbits each. These also developed localized areas of hemorrhage and necrosis in muscles, chiefly of the flat muscles or tendinous portions. One rabbit had also a few small hemorrhages in the acid-secreting portion of the stomach.

The primary culture in glucose-brain broth of the slightly hemolyzing streptococcus from the muscle lesions of one of these rabbits was injected into

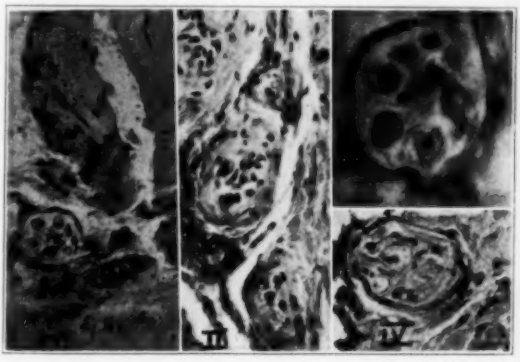

Fig. 5.—(I) Section showing slight round-cell infiltration and complete plugging of a small blood vessel from hyperplasia of endothelial cells in the tendinous portion of an excised section of muscle in a case of chronic myositis and arthritis deformans. Hematoxylin and eosin (X 200). (II) Obliteration of blood vessels in the tendinous end of a muscle in a case of chronic myositis and periarthritis. Hematoxylin and eosin (X 200). (III) Obliteration of blood vessels from hyperplasia of endothelial cells in a muscle in a case of chronic myositis and arthritis. Hematoxylin and eosin (X 700). (IV) Obliteration of a blood vessel in another field of section shown in Figure 5, I. Hematoxylin and eosin (X 200).

two rabbits, and the streptococcus from the blood of another animal was injected into one rabbit. The first two rabbits were well when chloroformed forty-eight hours later. Both had lesions in muscles. The third rabbit died in twenty-four hours from an overwhelming infection, with marked cloudy swelling of the muscles and viscera, and a few localized hemorrhages in the muscles of the back.

As a control experiment on the importance of injecting bacteria from the focus of infection one rabbit was injected with a suspension in salt solution of the bacteria from the anterior part of the mouth, and around the teeth.

and two rabbits were injected with the primary culture of these in glucose-brain broth. The blood-agar plates contained a few hemolyzing streptococci, many staphylococci and *Micrococcus catarrhalis*. The rabbit injected with the suspension, which contained many times the number of bacteria contained in the tonsillar suspension, and one of the two animals injected with the primary culture, had no lesions, while the third rabbit had a few lesions in the flat muscles on the left side of the thorax.

About ten days after the attack of myositis, the patient, without indiscretion in diet, became nauseated and vomited several times during the night. She ate very little the next day because of distress in her stomach. For about a week after this attack, she complained of heaviness and distress, amounting to distinct pain, in the epigastrium, usually from two to four hours after meals. Sodium bicarbonate gave relief. There was no fever, no leukocytosis, and no tenderness in the region of the gallbladder. The gastric symptoms disappeared entirely in about ten days.

Two days after the beginning of this attack, one rabbit was injected with a small amount of secretion expressed from the tonsil tissue. The amount was so small that it did not produce noticeable turbidity in 2 c.c. salt solution in which it was suspended for injection. The rabbit appeared to be well when chloroformed forty-eight hours later. It showed a number of small hemorrhages in the cardiac end of the stomach, and no lesions elsewhere.

The following day two rabbits were injected, one with 2.5 c.c., the other with 5 c.c. of the primary culture in glucose-brain broth from the tonsil tissue. Both appeared to be well when anesthetized two days later. Both had small hemorrhages in the stomach, most numerous in the pyloric end and along the lesser curvature. There were no other lesions.

An exactly similar experiment was performed at the same time as a control with material from the tonsil tissue from a patient having acute localized myositis (Case 1035). None of these three animals had lesions in the stomach, but all three had lesions in muscles.

In order to determine still further the importance of the peculiar infecting power which the bacteria seemed to acquire in a focus in the causation of symptoms in this patient, two rabbits were injected, one with the small amount of pus expressed from the tonsil, the other with the primary culture, two weeks after she recovered from the gastric attack. Both animals were well when chloroformed forty-eight hours later. There were no lesions in the stomach or duodenum, and none in the muscles with the exception of edema and a small group of hemorrhages, which had the appearance of a bruise, in the inner aspect of the knee in the one injected with the culture. This was in sharp contrast to the findings in four rabbits injected on the same day and with the same material from two cases (Cases 622 and 1035) of mild myositis, in which all of the animals had lesions of muscles, but no lesions elsewhere.

Case 6.—The patient [Case 1035], a laboratory technician, aged 20 developed a typical attack of stiff neck following an attack of tonsillitis in December, 1917. There were stiffness and pain on movement of the muscles of the right side of the neck, marked tenderness at the attachment of the muscles to the transverse process of the fifth cervical vertebra, and enlarged, slightly tender lymph glands just anterior to the margin of the right trapezius. There was no tenderness elsewhere and little or no fever. The tonsils were moderately enlarged, and a small amount of pus was expressed from the pole of each.

The pus, when plated directly, as well as the cultures in broth on plating, showed almost a pure growth of slightly hemolyzing streptococci. The emulsion in salt solution of a small amount of the pus, so small as to produce only slight turbidity, was immediately injected into a half-grown rabbit. The animal became lame in the right hind leg in two days, and was chloroformed. It had large swelling, edema and hemorrhage of the muscles of the anterior and outer aspect of the right tibia and along the tendo achillis around the

right ankle, and a moderate number of small hemorrhages in the intercostal muscles on the left side and in the deeper muscles on the left side of the neck. The joint fluids were clear, and no lesions of viscera or nerve trunks were found.

Two additional rabbits were injected at the time of this attack, one with the primary culture of the pus in glucose-brain broth, and the other with a pure culture of the streptococcus in this medium after plating on blood-agar. Within forty-eight hours both animals showed a marked disinclination to move about, and were chloroformed. Both had marked, localized lesions, chiefly in flat muscles and tendinous ends, turbidity of joint fluid and hemorrhages around one knee joint, and a few embolic areas in the kidneys.

January, 1918, the patient had an attack resembling cholecystitis, which followed a severe fall on the right hip on the ice. February 17, the pain in the

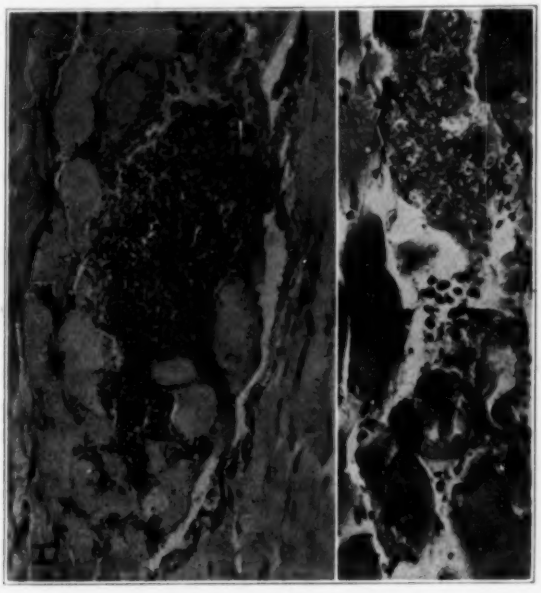

Fig. 6.—(I) Section of a whitish lesion, like those shown in Figure 1, (I), in the muscle of a rabbit injected with the streptococcus from the tonsils in Case 904. Hematoxylin and eosin (X 100). (II) Slight leukocytic infiltration and edema between the muscle fibers and the myocardium of a rabbit injected with streptococcus from the tonsils, after one animal passage, in a case of myositis and myocarditis (Case 601). Hematoxylin and eosin (X 200).

region of the gallbladder recurred during the night. Five days later, after she had completely recovered from the second attack of pain, two rabbits and one dog were injected with the primary culture from the tonsils in tall columns of glucose broth. The animals were chloroformed forty-eight hours later. No lesions were found.

April, 1920, the patient developed, from no apparent cause, pain and tenderness in the left arm, midway between the shoulder and elbow joint. She was nauseated and dizzy for two days. The left tonsil contained a large crypt filled with yellowish-gray, foul smelling, cheesy material. Firm pressure dis-

lodged the plug and a full minim of grayish pus escaped. A small part of the latter was suspended in 2 c.c. salt solution and injected into a medium sized rabbit. The rabbit was well when it was chloroformed the following day. It had a small hemorrhage in the tendinous end of the muscles of the posterior aspect of the left leg, and a large hemorrhagic area in the muscles of the anterior and inner aspect of the upper end of the right tibia.

On the basis of these experiments tonsillectomy was advised, and the tonsils were removed in July, 1920. The patient was free from the attacks resembling cholecystitis and from myositis until Feb. 1, 1921, when she developed pain in the muscles of the left arm and right scapula, following sore throat. Examination revealed infected tonsil tissue, with one deep crypt containing pus at the lower end of the tonsil scar on the right side. No experiments were performed at this time. The symptoms referable to the muscles disappeared in a few days, but the right tonsil area remained tender and painful on swallowing. Feb. 18, 1921, she developed a sharp pain, especially on exertion and pressure on the inner aspect of the muscles above the left knee, and the following day stiffness and soreness of the muscles of the deeper layers of the right side of the neck. This was not associated with fever or general illness, and the pain in the muscles lasted only two or three days. February 19, the small amount of secretion expressed from the tonsil tissue was suspended in salt solution and injected into a rabbit weighing 1,000 gm. The animal appeared to be well forty-eight hours later when it was chloroformed. An area 1 cm. in diameter in the muscles of the inner aspect of the left thigh just above the knee joint showed a group of hemorrhages, whitish lesions, edema and infiltration. Similar, but smaller, localized lesions were found in the muscles around the right elbow joint and the left side of the thorax. The two rabbits injected with the primary culture from the tonsil in glucose-brain broth appeared to be well when they were anesthetized forty-eight hours after the injection. Both animals had localized lesions in muscles, but no lesions elsewhere. The cultures of the tonsillar secretion on blood-agar, the primary culture in broth, and the culture from the muscle lesions contained green-producing streptococci and staphylococci.

February 28, the patient awakened with pain in the throat, which increased on swallowing, and during the day she developed pain over the left shoulder blade, which was aggravated on muscular effort. This disappeared in a few days. On the morning of March 1, while the pain in the muscles was still present, one rabbit was injected with a suspension in salt solution of the small amount of pus expressed from the remnant of tonsil tissue. The rabbit appeared to be well when it was chloroformed forty-eight hours later. Groups of hemorrhages in the muscles over the left shoulder blade and in the superficial fascia over the back were found. Late on the same day, one rabbit was injected with 5 c.c. of the primary culture of the tonsil pus in glucose-brain broth. The rabbit appeared to be well when it was chloroformed forty-eight hours later. A few small hemorrhagic lesions were found in the muscles of the left fore extremity, especially in the tendinous ends of the muscles around the elbow joints.

Details of the More Important Cases in the Myositis and Arthritis Group (Table 2)

Case 7.—Mr. F. K. [Case 536], aged 27, came to the clinic, Nov. 24, 1915, complaining of rheumatism, which he had had for three years. It began with pain in the right shoulder and right knee; later it extended to the left shoulder and left knee, and occasionally to the second finger of both hands. There was also aching of the muscles of the back, arms and legs. The trouble in the joints, except in the right shoulder, had stopped, but the pain in the muscles was constantly growing worse. Examination revealed periarthritis, and lesions, chiefly of the tendinous ends of muscles. The tonsils contained much caseous material and were removed. After the removal of the tonsils, the

patient had less muscular pain. A year later he came back. The muscle aching had disappeared, but he had periodic attacks of muscle soreness, associated with sleepiness and tired feeling. The roentgen-ray examination of the teeth was negative. The patient was not referred again to the laboratory, but was given general treatment and dismissed.

The rabbit injected with the pus from the tonsil seemed to be ill when it was chloroformed forty-eight hours later. Examination revealed a moderate number of edematous, necrotic lesions in the muscles of the extremities, neck and shoulders, a few hemorrhages in the papillary muscles of the left ventricle and in the tricuspid valve, and slightly turbid fluid in the knee joints. Cultures from the lesions in the muscles contained *Staphylococcus aureus*.

The two rabbits injected with the primary culture in ascites-glucose broth had marked lesions in the muscles and myocardium, and one rabbit had in addition a few embolic lesions in the kidneys, and turbid joint fluid. The primary culture from lesions in the muscle of one of these, containing staphylococcus, was injected into one rabbit. It seemed muscle sore when it was chloroformed four days later. A few muscle lesions, multiple suppurative arthritis, a few embolic lesions in the kidneys, and cystitis with marked infiltration of the bladder wall were found.

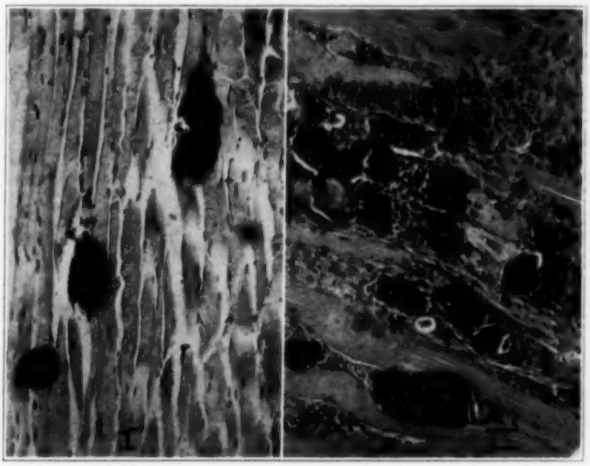

Fig. 7.—(I) Section of muscle in a rabbit six days after injection of streptococcus from the muscle in the case of calcifying myositis (Case 130). Note the marked deposit of calcium in sharply circumscribed areas. Hematoxylin and eosin (X 80). (II) Section of muscle of rabbits shown in Figure 1, I. Note the disintegration of the muscle fibers, leukocytic infiltration, and moderate deposit of calcium in the necrotic areas. Hematoxylin and eosin (X 100).

Case 8.—A physician [Case 552], aged 51, had suffered from repeated attacks of muscular and neuralgic disturbances, lumbago and sciatica, for twelve years. He had had rheumatic fever when he was a child. He had always had more or less sore throat, which had been worse for the last three years. Three years before examination he had had pericarditis, and eighteen months before, following an attack of sore throat, he had had acute arthritis of the right knee, and pain in the muscles of the neck and left shoulder. Roentgenograms of the knee and spine were negative. Both tonsils contained a few small pus

pockets in their depths. The tonsils were removed and an emulsion was made of them and cultured. The growth of green and slightly hemolyzing streptococci which was obtained was injected into two rabbits, and produced in both animals hemorrhagic lesions in the muscles of the back and shoulders, and in one rabbit a few lesions in the thigh and arthritis of the knee joints (Table 2).

The patient has reported that within two months he became free from all rheumatic disturbances, and that he had gained in weight. Five years after his tonsillectomy he wrote that his former symptoms had not returned.

CASE 9.—A middle aged physician [Case 622], had had repeated attacks of acute tonsillitis for many years. Following two such attacks, while he was in the medical school, hydrops of the left knee developed. He had repeated attacks of lumbago, usually with mild myositis, involving chiefly the flat muscles around the back, neck and thorax. Because of these attacks, and because foul smelling, cheesy plugs kept forming in the tonsils, tonsillectomy was performed. After this there was freedom from the symptoms for about two years; then followed a loss of endurance, a prolonged low grade arthritis which involved the small joints of the hands, a number of attacks of myositis, irritability of the bladder, presumably due to infection of the prostate, and a troublesome hyperchlorhydria. The joint and muscle symptoms became worse following inflammation of the tonsil tissue in the base of the left tonsillectomy scar. These symptoms, and those referable to the bladder, disappeared promptly after the removal of the remnant of the left tonsil, which contained a small abscess filled with foul smelling pus. The streptococcus from this abscess and from shreds in the urine produced arthritis, chiefly of the peripheral joints, and myositis in the four rabbits injected.

About two years later, the patient had an attack of myositis and subacromial bursitis, a typical attack of so-called "painful" shoulder, and the symptoms referable to the bladder returned. They were never severe, but persisted for a number of months; those caused by the bursitis were severe at first and were associated with tender deltoid and triceps muscles on the affected side. The symptoms grew milder during several months, and then persisted practically unchanged for nearly a year. The nine rabbits injected with the streptococcus from the shreds in the urine developed myositis and subacromial bursitis. A devitalized molar, believed to bear a causal relationship, was extracted, even though the roentgenogram was negative. A small granuloma was found situated so that its demonstration was impossible in the roentgenogram. The granuloma was incubated a short time, a pure culture of slightly hemolyzing streptococcus was isolated, and a large colony of streptococci was found near the apex in intimate relation to the blood vessels. The symptoms referable to the shoulder disappeared promptly, a result strikingly similar to the results reported by Evans in this painful affection. No joint or muscle symptoms developed until seventeen months later, when a mild attack of myositis occurred, and symptoms of hyperchlorhydria again became troublesome. Examination at this time revealed three devitalized teeth with defective root canal fillings. Two of these, the two left lower bicuspids, had been devitalized eighteen years before; the third, a right lower molar, had been devitalized six years before. The roentgenogram of the first two showed increased density of the structures around the roots, and one showed a faint line of rarefaction over the apex, while the molar appeared to be normal. The material, which was removed in a sterile manner from the canals of the first two teeth, contained pure cultures of a green-producing streptococcus. Antiseptic treatments efficiently sterilized the canals, but it was feared that the tissues beyond could not be thus sterilized, and the three nonvital teeth were extracted under sterile precautions. Cultures of the apices showed streptococci in each. These cultures were injected into three rabbits. All three developed characteristic lesions. The results are illustrated in the following experiment:

A rabbit weighing 1,330 gm. was injected intravenously Oct. 5, 1918, with 8 c.c. glucose-broth culture from the roots of the second left lower bicuspid. October 6, at 7:30 a. m., the animal was found extremely weak, scarcely able to walk. At 10 a. m. it was found lying quietly on its side, and it died without convulsion. Necropsy revealed a moderate number of hemorrhages in the muscles of the back and hips, and a large hemorrhagic, edematous area opposite the root of the right lower incisor. The peridental membrane of the left lower incisor was edematous and hemorrhagic throughout, while that of the right was hemorrhagic and edematous at the apex. The pupils were edematous and hemorrhagic. The other teeth appeared to be normal. There were a large number of submucous hemorrhages in the stomach and appendix and one hemorrhage in the tricuspid valve. The joints were normal, as were also the myocardium, kidneys, spleen, liver and other organs. The blood-agar plate of the blood contained no growth, while the blood-agar plate of the hemorrhagic

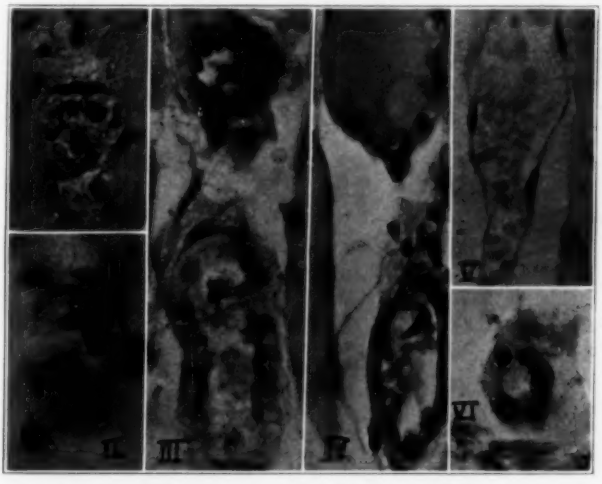

Fig. 8.—(I) Cross section of capillary showing aggregation of leukocytes within the lumen adjacent to the lesions in the muscle shown in Figure 6, (I). Hematoxylin and eosin (X 500). (II) Diplococcus in the wall of a capillary showing marked swelling of the endothelial cells in the muscle of a rabbit one day after five daily injections of liquor formaldehydi-killed streptococci from Case 904 after one animal passage. Giemsa (X 1000). (III) Cross section of an arteriole and a venule in the muscle adjacent to lesions in rabbit shown in Figure 1 (I). Note the marked swelling of the endothelial cells. Hematoxylin and eosin (X 500). (IV) and (V) Sections of normal arteriole, venule, and capillary of muscle in Figure 8 (III), but remote from lesions. Note the absence of swelling of the endothelial cells. Hematoxylin and eosin (X 500). (VI) Marked swelling of the endothelial cells in a capillary in the muscles of rabbit shown in Figure 1(I). Hematoxylin and eosin (X 500).

pulp contained a moderate number of slightly hemolyzing and green-producing streptococci.

Immediately following the extraction of his teeth, the patient developed a sharp attack of myositis of the muscles of the lower right thorax, after which there was a noteworthy freedom from symptoms for six months. A similar attack, though milder, involving the muscles of the neck and shoulder, occurred

following infection of the gum, which became painful from pressure of an improperly fitting denture. With the correction of this difficulty, the myositis again subsided. Since then, the patient has had unusually good general health and has been quite free from myositis attacks and from symptoms of hyperchlorhydria.

During the latter part of February, 1921, the patient developed an attack of nasopharyngitis, followed by myositis of the muscles of the left lower thorax, the left shoulder, and the left side of the neck. During this attack two rabbits were injected, one with a suspension of the secretion from a swab from the nasopharynx, the other with the primary glucose-brain-broth culture. Both were well when chloroformed forty-eight hours later, and both had hemorrhagic lesions in muscles, chiefly of the thorax and shoulder.

The incidence of lesions found in the seventeen rabbits injected is given in Table 2.

Improvement followed the removal of foci of infection in this patient on four widely separated occasions, and the presence of bacteria in the foci removed, which had specific localizing power, was demonstrated on three occasions.

Case 10.—A laboratory worker [Case 913], aged 37, had pain and stiffness in the left knee, noticeable in walking downstairs, or in rising from a sitting position. After nine months, this spontaneously disappeared. About two months later, the right knee became affected to a greater extent, with increasing severity. The painful area extended up the muscles of the inner aspect of the thigh, and occasionally there was pain in the muscles around the shoulders and hip joints, and intermittent slight looseness of one or two teeth. Two ulcerated teeth had been extracted, but without beneficial results.

Examination revealed swelling and tenderness in the subpatellar bursa. The tonsils were red, but small. The anterior pillars were adherent and tightly covered a large part of both tonsils. They were uncovered with difficulty and four or five crypts were found packed with white cheesy material and pus, which was expressed.

Two rabbits were injected with the suspension in salt solution of this pus, and as a control two rabbits were injected with a suspension of a comparable amount of pus expressed from the tonsils of a person (Case 912) who was well at the time. The four animals appeared to be well when they were chloroformed forty-eight hours later. The two animals injected with the pus from the patient's tonsils had lesions in muscles, periosteum and joints. One animal had a few small red lesions in the tendinous portions of the muscles of the shoulder and the bursa just below the patella, a hemorrhagic area below the right lower cuspid, and a periostitis below the right eye. The other had edema of the tissues surrounding one knee joint, circumscribed areas of hemorrhage in the periosteum of the upper and inner aspect of the tibia just below the epiphyseal line, hemorrhagic lesions in the inner aspect of the periosteum adjacent to the elbow joint and in the capsule of the hip joint, a number of linear hemorrhages in the quadriceps tendons, and an area of hemorrhage in the muscle overlying the hip. The two rabbits injected with the pus from the tonsils of the person who was well did not show lesions.

The following day two rabbits were injected with the primary culture of the pus from the patient's tonsils, and two others were injected with identical amounts of the culture of the pus from the tonsils of the normal control. Smears and plating of both cultures revealed almost a pure, short chain, slightly hemolyzing streptococcus. The rabbit injected with the smaller dose (the growth from 15 c.c.) of the control culture did not develop lesions; the one receiving the larger dose (30 c.c.) had a few small hemorrhages in muscles and slight turbidity of joint fluid, which was sterile. Of the two rabbits injected with the culture from the patient, the one rabbit receiving the smaller amount developed hemorrhagic lesions, especially of the ligaments around the joints, the

subpatellar bursae and intracapsular fat, and the intercostal muscles, a hemorrhagic area in the stomach, and a few embolic streaks in the kidneys. The lesions in this rabbit were more pronounced than those in the control rabbit injected with the larger dose. The rabbit injected with the larger dose died of an overwhelming infection within eighteen hours, without developing focal lesions. A third rabbit was then injected with the same culture. It survived for forty-eight hours, and developed lesions in the tendinous portion of the muscles surrounding the right elbow, in the intercostal muscles, and in the body of the triceps.

As a further control of this work, cultures were made from single colonies taken from aerobic blood-agar plates seeded with the tonsil pus culture of the patient. Hemolytic and green-producing streptococci were used. The animals injected with these cultures gave negative results, which bears out our general finding that not every streptococcus will cause these muscle lesions, and that the streptococcus which will cause these lesions either does not grow or loses its peculiar infecting property when grown on aerobic blood-agar plates.

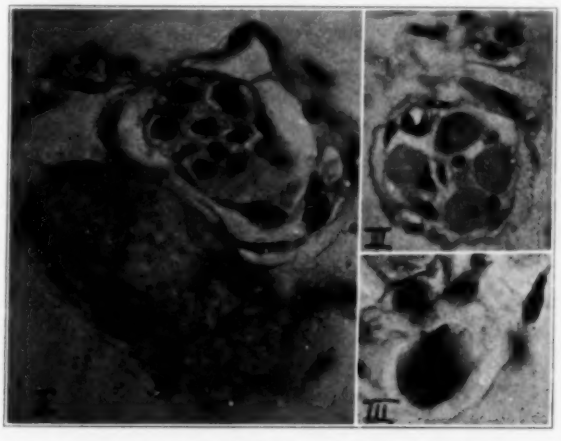

Fig. 9.—(I) Cross section of arteriole, venule, and nerve fiber of a rabbit one day after five daily injections of liquor formaldehydi-killed streptococci from Case 904. Note the marked proliferation of the endothelial cells in the arteriole. Hematoxylin and eosin (X 500). (II) Cross section of nerve fiber and terminal sensory organ in muscle of rabbit referred to in Figure 9 (I). Hematoxylin and eosin (X 500). (III) Diplococcus in partial focus within the swollen cells of the sensory organ shown in Figure 9 (II). Giemsa (X 1000).

The culture made from the muscle lesions which developed in the rabbit receiving 15 c.c. of the patient's tonsil pus culture was injected into three rabbits. Two rabbits received 2 c.c. doses of the broth culture and one a 5 c.c. dose. The first two rabbits had hemorrhagic lesions in the muscles. The third rabbit had a few lesions in the muscles and some in the periosteum.

On the basis of these findings, the tonsils were removed, and, although small, a number of abscesses were found in their depths. Injection into animals of a salt solution emulsion, and the resulting cultures, gave results almost identical with those obtained with the tonsil pus and cultures

Altogether twelve animals were injected with organisms obtained either directly from the tonsils or by animal passage. One of these animals died of an overwhelming infection in less than twenty hours. All of the others had

lesions of the muscles of a uniform type, six also had lesions in the periosteum and six joint lesions of the subpatellar bursa. Of these eleven rabbits, one had, in addition, endocarditis, one had a stomach lesion, two had kidney lesions, two had skin lesions, and two had enlarged lymph glands (Table 2).

Four rabbits were injected with the material obtained from a normal person whose tonsils were in a worse condition than the patient's tonsils. Three of these rabbits were entirely negative. The fourth, which received more than twice as great a dose as any of the rabbits of the first series, developed a few lesions in muscles and periosteum, and a slight turbidity in the knee joint fluid. The organisms, although not highly virulent, showed evidence of being more virulent than those obtained from the normal control.

The patient's serum agglutinated specifically her own strain in the first and second culture generation and the strain from the muscle lesion in higher dilutions (1:100) than did the serum from the normal person, but not the control strain in the first and second culture nor the hemolytic streptococcus from her own tonsils.

A few days after the patient's tonsils were removed, the pain in the muscles and subpatellar bursa had disappeared almost completely; only a slight tenderness in the joint remained. After a few weeks there was some return of the trouble, but the patient recovered entirely within two years, and has remained well for several years.

DETAILS OF THE MORE IMPORTANT CASES IN THE MYOSITIS AND NEURITIS GROUP (TABLE 3)

CASE 11.—Miss B. C., [Case 571] aged 22, had never been strong, and was very nervous. She had a dry hacking cough, sometimes raising blood-stained sputum, with pain in the muscles of the chest and around the shoulders, numbness of the right arm and nausea, especially when she coughed. She was constipated, suffered from gas in the bowels, and had had right-sided headaches since childhood. She had lost 10 pounds in weight in the last year, and weighed 98 pounds at the time of examination.

Physical examination revealed moderate hypertrophic rhinitis, hypertrophied adenoids, infected tonsils and a cloudy right antrum. Roentgenograms of the chest and the colon were negative. Nasal washes were prescribed, and tonsillectomy was performed. The patient improved markedly soon after the tonsillectomy. The excised tonsils were cut into small pieces and were found to contain pockets filled with foul smelling pus. The emulsified tonsil was cultured and a growth consisting mostly of streptococci was obtained. This was injected into four rabbits and one mouse. All of these animals developed muscle lesions, three chiefly in the muscles of the shoulders, fore legs and thorax; two of the rabbits had hemorrhage and edema in the sheath of the popliteal nerve (Fig. 3, II).

CASE 12.—A man, [Case 601] aged 46, a painter, had been subject to muscular rheumatism for several years. Since February, 1916, he had had attacks of pain across the chest in the region of the heart, radiating to the back, so severe that he felt like lying down. For a week previous to his examination, the pain had been more or less constant. He was short of breath, especially at the time of the attacks, and he fainted on several occasions. His habits were good.

At the time of examination his cheeks were flushed, his pulse 64, and irregular; there were no heart murmurs. He had marked pyorrhea, and the gum edges presented a bluish discoloration. There was no granular degeneration of the erythrocytes. The Wassermann reaction was negative. A cardiogram showed a pulse rate of 50, marked impairment of conduction, and a damaged right His bundle. The urine examination was negative. He had not had tonsillitis, and the tonsils were fairly clean. He had a slight laryngitis.

A culture of the pus from the pyorrheal pockets contained a moderate number of colonies of green-producing and slightly hemolyzing streptococci. Roentgenograms of the teeth revealed alveolar abscesses in three. These teeth were extracted, and prophylactic treatments for the pyorrhea were instituted. The cultures made from the pus from the pyorrheal pockets and the alveolar abscesses, both of which were extremely foul, contained a moderate number of colonies of green and slightly hemolyzing streptococci, a few gram-positive bacilli, and staphylococci.

Four and one-half months after the extraction of the teeth and the treatment for pyorrhea, the patient was reported to be feeling better; there was less dyspnea, but at times he still had palpitation and spells of weakness, although no fainting spells as formerly. Two years later the improvement was still manifest.

In this case thirteen animals were injected, either with the pus from the pyorrheal pockets or with the streptococci from the lesions produced on animal passage. The incidence of lesions is given in Table 3. Seven of these animals

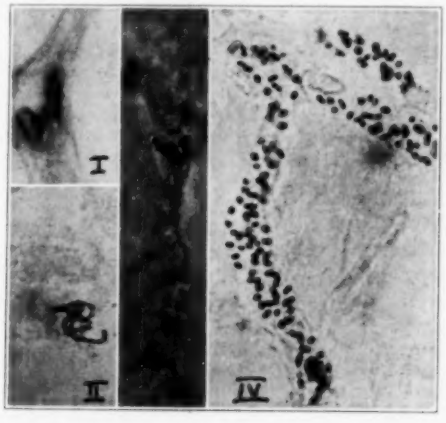

Fig. 10.—(I) Chain of two diplococci in the section of human muscle shown in Figure 4 (I). Gram-Weigert (X 1000). (II) Chain of streptococci in the area of leukocytic infiltration shown in Figure 4 (II). Gram-Weigert (X 1000). (III) Diplococci in the area of leukocytic infiltration shown in Figure 6 (I). Gram-Weigert (X 1000). (IV) Large number of gram-positive diplococci in capillaries of a hemorrhagic area in the quadriceps tendon of a rabbit two days after injection with the streptococcus from the tonsils in a case of periarthritis and myositis (Case 913). Gram-Weigert (X 1000).

had lesions in the muscles. Ten (80 per cent.) had pericardial, myocardial, or endocardial involvement. One had a hemorrhagic lesion in the region of the bundle of His; one had a mural vegetation in the region of the bundle of His, and one, a dog, which developed a gallop rhythm of the heart, had a lesion in the left ventricular side of the septum just beneath the aortic cusp, probably in the left limb of the His bundle. Two of the animals had lesions in the aortic arch. One rabbit had a moderate number of hemorrhages throughout the length of the left vagus nerve. The right sympathetic nerve and the right cervical sympathetic ganglion were hemorrhagic and edematous. Sections showed a large number of gram-staining diplococci in the hemorrhagic ganglia. A few hemorrhages were found also in the cervical plexus.

The localization and lesions produced in this case were quite unique. The gallop rhythm, the lesions in the bundle of His and sympathetic nerves and ganglion are especially noteworthy.

Case 13.—Mr. P. W. [Case 2291], aged 49, had had recurring attacks of appendicitis until his appendix was removed in 1910. He came to the Clinic Sept. 18, 1918, complaining of nervousness, loss of mental and physical endurance, attacks of fluttering of the heart at night, pain in the back, and a daily afternoon rise of temperature. The pain in the back was dull, aching, and deep in the muscles of the lumbar region, often radiating into the thighs. The pain was worse when he exercised; he could walk only several blocks at a time. His temperature rarely went above 99.6 F. He had seen many physicians; some of them diagnosed incipient pulmonary tuberculosis because of the temperature and the finding of a few râles in the lungs; others suspected, but did not demonstrate, chronic focal infection. He had an attack of pneumonia lasting three days nine months before coming to the Clinic.

The man was well nourished, but markedly neurotic. The vasomotor tone was below par. The thyroid was palpable on both sides, indicating a small adenoma. The pulse was regular, and the heart tones were clear. A few râles were heard over the apices of both lungs. Blood culture and blood Wassermann reactions were negative. The blood pressure, the urine, the stool, and the blood count were normal. The metabolic rate on three occasions was —23, —19 and —2, respectively. The tonsils appeared to be normal on inspection, but on pressure a moderate amount of pus was expressed.

One rabbit was injected with a suspension of a part of this pus, and on the following day another rabbit was injected with the primary culture in glucose-brain broth. Both animals appeared to be muscle sore when they were chloroformed three days later. The first rabbit had a number of circumscribed areas of hemorrhagic edema in both psoas muscles. The lumbar nerves passed through some of these areas, and hemorrhages were found in the sheath of two of the nerves remote from the lesions in the muscles. Similar, but smaller, lesions were found in the muscles in the lumbar region and thighs. The second rabbit showed almost identical findings, but the lesions were relatively more marked in the muscles of the back and neck, while lesions of nerve sheaths were not found remote from the hemorrhagic areas in the muscles. The cultures of emulsions from the hemorrhagic muscles showed *Staphylococcus aureus* in both rabbits, and no streptococci. Cultures of the blood, joint and cerebrospinal fluids, kidneys, spleen and normal muscle were sterile.

On the basis of these findings, tonsillectomy was performed. The extirpated tonsils contained several large abscesses. Following the removal of the tonsils, the fever and the pain in the muscles of the back largely disappeared. The nervousness and lack of endurance persisted for some months, but after the administration of thyroxin, on the advice of Dr. H. S. Plummer, improvement in these symptoms occurred.

DESCRIPTION OF SYMPTOMS AND LESIONS IN ANIMALS

The animals injected with streptococci from the patients with generalized myositis usually developed numerous widely disseminated lesions, while those injected with strains from patients with localized areas of myositis usually developed only localized lesions in the muscles. The former often presented quite characteristic symptoms from which the existence of myositis was frequently predicted. The muscles appeared tender to touch. The animals sat around quietly, with ruffled fur, sometimes resting the nose on the floor of the cage. They were disinclined to move and when made to do so, walked about as if muscular effort

caused pain and discomfort. They lost markedly in weight and often became very weak. The rabbits showing only localized lesions were usually free from demonstrable symptoms, and lost little or no weight. In some instances in both groups of animals, however, the discomfort from muscular effort noted during life could not be explained satisfactorily by the few gross lesions which were found in the muscles after death. In these animals, lesions of nerves, or hemorrhagic areas of muscles or fascia surrounding nerves were often demonstrable. (Figs. 2, I, III, 3, I, II).

The lesions were of two main types. One type consisted of numerous small linear or oblong hemorrhages (Fig. 1, I); the other consisted of equally numerous small linear, grayish white, necrotic streaks (Fig. 1, II). The former was, in general, the early stage of the latter, and both types were often present in the same animal in varying proportions. The time when the hemorrhagic lesions became necrotic varied greatly, depending to some extent on the size of the dose injected, but most of all on the strain of streptococcus. Following the injection of some strains, only necrotic areas were found, even as early as twenty-four hours after injection; with other strains, only hemorrhagic areas were found, and these often disappeared without becoming necrotic. The lesions obtained with staphylococci were quite different. Suppuration frequently occurred, and the surrounding edema and hyperemia were more pronounced. The small hemorrhagic and necrotic lesions due to streptococci were often widely distributed throughout the skeletal muscles. They were nearly always more numerous in the flat muscles of the trunk, along the tendinous partitions, and in the tendinous ends of the muscles of the extremities. This picture was in sharp contrast to that following the injection of the strains from cases of localized myostitis. Here disseminated lesions rarely developed, but instead localized areas of large and small hemorrhages, associated early with little or no edema and infiltration, while later the edema, infiltration and necrosis often became marked. Softening, with pus formation, was rare, but it occurred more often in the animals with large localized lesions than in those with small, widely disseminated lesions.

The microscopic findings were very interesting. The lesions usually developed between the muscle fibers, and consisted, first, of extravasation of red blood corpuscles, dilatation of adjacent capillaries, and swelling of the muscle fibers with disappearance of the transverse striations as interstitial edema and leukocytic infiltration became manifest. Later, in the larger lesions, the muscle fibers became fragmented and necrotic as leukocytes and other cells became numerous. The amount of cellular infiltration varied from large areas of infiltration (Fig. 6, I) to the aggregation of only a few cells between the muscle fibers (Fig. 6, II). Deposits of calcium salts occurred relatively early

in the necrotic areas (Fig. 7, II), especially in the rabbits after the injection of the streptococcus isolated from the muscle in the case of calcifying myositis (Fig. 7, I). A marked deposit of calcium was noted as early as forty-eight hours after the injection of this strain; whereas, after the injection of other strains when necrotic lesions were fewer, little or no calcification was noted. Areas of necrosis of considerable size appeared necessary for the deposit of calcium, a fact which seems to support Wells' theory of calcification.[17] There was usually no difficulty in demonstrating the organism in the lesions soon after injection (Figs. 8, II; 9, III; 10, III, IV), but later this was often difficult and sometimes impossible. The microscopic changes in the human muscles in acute myositis (Fig. 4, I, II) were similar to those produced experimentally. They consisted of extravasation of red blood corpuscles, interstitial edema, cellular infiltration, swelling and necrosis of the muscle fibers, with disappearance of transverse striations. The demonstration of streptococci was also possible (Fig. 10, I, II). The whole picture parallels quite closely the description by Körmöczi[18] of the microscopic findings in muscles in a patient who died of acute myositis caused by a streptococcus.

The sections of atrophic and tender muscles and periarticular structures in chronic myositis and arthritis showed relatively little cellular infiltration (Fig. 5, I), but showed a marked swelling of the endothelium and sometimes complete plugging of the blood vessels from proliferation of endothelial cells (Fig. 5, I, II, III, IV). Streptococci have been isolated by cultural methods from these excised tissues and demonstrated in the endothelial cells, but not without prolonged search.

Owing to these findings it was thought that a study of the changes in the blood vessels in the muscles of rabbits showing lesions might throw some light on the mechanism involved in the localization of the bacteria and the production of the lesions. The changes in the small blood vessels were found to consist of two quite distinct types, aggregation of leukocytes within the lumen (Fig. 8, I), and more rarely in the perivascular lymph spaces. This occurred commonly after the injection of strains from patients with acute myositis, who recover promptly. The demonstration of bacteria in the wall of these vessels was not accomplished, but in a few instances diplococci were found within the leukocytes. The small vessels following injection of the strains from chronic generalized myositis, especially after repeated injections of the dead organisms, presented a very different picture. There were few or no leukocytes even soon after injection. The reaction

17. Wells, H. G.: Text Book of Chemical Pathology, Philadelphia, W. B. Saunders Co., 1918, pp. 707.

18. Körmöczi, E. v.: Durch Streptokokkeninfektion verursachte Polymyositis (Polymyositis Streptomycotica), Centralbl. f. Bakteriol. **31**:688, 1902.

appeared to be chiefly endothelial. The endothelial cells lining the vessels were often markedly swollen (Fig. 8, III, VI), and diplococci were sometimes demonstrable (Fig. 8, II). Proliferation of endothelial cells containing deeply staining nuclei occurred in some arterioles, especially in prolonged experiments where repeated injections of liquor formaldehydi-killed streptococci were given (Fig. 9, I). The changes noted in the small blood vessels in response to the intravenous injection of bacteria recall the peculiar behavior of small blood vessels noted by Hooker [19] in the ear of the cat during life following the injection of hystamin and other substances.

During the microscopic study, attention became directed to what, at first, were thought to be cross sections of blood vessels in which the lumen was filled with endothelial cells, but which on closer study were considered to be peripheral sensory end organs (Fig. 9, II). The cells of these differed from swollen endothelial cells in being more nearly round, and in that nuclei were usually situated to one side. After prolonged search undoubted diplococci were found in these highly specialized nerve cells (Fig. 9, III).

LOCALIZATION OF DEAD STREPTOCOCCI FROM MYOSITIS

It was thought that the injection of the dead bacteria as a control might throw light on the mechanism of the production of myositis. Particularly suitable cultures, those in which the living organisms produced marked lesions in muscles, were used. Liquor formaldehyd was selected as the most desirable agent with which to kill the streptococci. One of us had found previously that liquor formaldehyd-killed pneumococci failed to autolyze. The agglutinability of typhoid bacilli killed with liquor formaldehydi remains unchanged for a long time.

After growing the streptococci in tall columns of ascites-glucose broth for from eighteen to twenty-four hours, there were added 0.5 c.c. liquor formaldehydi (40 per cent. strength) for each 100 c.c. of the culture. This was thoroughly mixed and allowed to stand at room temperature for twenty-four hours, when sterility tests were made. The dead bacteria were then removed by centrifugation, and the sediment, which was drained dry, was suspended in 0.85 per cent. salt solution so that 1 c.c. contained the growth from 15 c.c. of the broth culture. From 0.5 to 10 c.c. of this suspension, representing the growth of from 7.5 to 150 c.c. of broth, were injected into rabbits weighing from 1,050 to 2,150 gm. In most of these animals repeated daily injections, as high as six, were given. The animals were sacrificed usually in from one to ten days after the last injection. The effect of dead streptococci from five cases of myositis (Cases 130, 369, 567, 601, and 904) was studied in eleven rabbits. Lesions similar in location and distribution to those obtained with the corresponding live bacteria were obtained in eight animals following injection of the strains from four cases. No lesions were found in the other three, all of which were injected with small doses.

In the case of dental neuritis and cervical myositis (Case 567) the three rabbits had lesions in the flat and tendinous partitions of muscles and dental pulps. One of these was injected once with the dead streptococci from the

19. Hooker, D. R.: The Functional Activity of the Capillaries and Venules, Am. J. Physiol. **54**:30, 1920.

primary growth of the excised muscle, one on four occasions with this strain in the second culture from a single colony on blood-agar, and one with the dead streptococci from the primary culture from the infected tooth pulp. The Berkefeld filtrate of the supernatant broth was injected intravenously daily into one rabbit for three days in 12 c.c. doses. It remained well and was anesthetized for examination on the fourth day. No lesions were found. The lesions following the injection of the dead organism were less numerous and smaller than those following the injection with the corresponding live culture, even though the dose of the dead bacteria was much larger. The lesions consisted of small hemorrhages with little edema and infiltration. In some areas dilatation of blood vessels was found microscopically, in others, constriction, with swelling of endothelial cells. In some instances, the vessels were partially or completely filled with endothelial cells. Intravascular and interstitial leukocytic reactions were slight or absent. The changes in the muscle fibers consisted chiefly of swelling, disappearance of the striations, occasionally of fragmentation, and at times of beginning fatty degeneration.

The demonstration of the streptococci in the tissues was difficult. Most of the bacterial cells were disintegrating and had lost much of the gram stain. Unmistakable diplococci, sometimes in short chains, were found within swollen endothelial cells lining the small blood vessels in the areas of hemorrhage (Fig. 8, II), between the muscle fibers, and within what appeared to be sensory end organs (Fig. 9, III).

DISCUSSION AND SUMMARY

Localized infections around teeth and in tonsils were demonstrably present in nearly all of the twenty-eight patients studied. Improvement in symptoms, often striking, occurred in all but one of the twenty-five patients from whom foci were removed. In the twenty-four patients in whom improvement did occur the focus was shown to contain bacteria which tended to produce lesions in the muscles of animals, while in the one patient in whom improvement did not occur the bacteria failed to produce lesions. In some patients recurrence of myositis or only partial recovery was found to be due to defective tonsillectomies, to inadequate dental operations, to failure in recognizing the existence of foci in teeth, or to the development of new localized areas of infection. Therefore, localized infections in tonsils, teeth and elsewhere, in the patients studied appeared to play an important part not only in the etiology of pure myositis, but also in associated conditions, such as myositis and arthritis, myositis and neuritis, nontraumatic lumbago and myocardial degeneration. In the light of these facts, foci of infection apparently not only make a forced relationship between the bacteria and their products and the mechanism of resistance of the host, but, it would seem, afford the conditions favorable for the microorganism to acquire peculiar infecting powers.

The elective affinity of the streptococci from these localized infections was so marked, that the organisms not only tended to localize and produce lesions in muscles, but the location of the lesions often approximated that noted in the patient. When the affection of other structures, such as joints and nerves, appeared as a secondary factor in the patient's symptomatology, lesions were also found in these struc-

tures in the injected animals, and the percentage of animals showing them was proportionately less than the percentage of animals showing muscle lesions. In pregnant rabbits localization was found to occur not only in the muscles of the parent rabbit, but also in the fetuses.

The localization in the myocardium, especially in the bundle of His, and the concomitant development of arhythmia of the heart following the injection of cultures from pyorrheal pockets, in the case of impaired myocardial conduction, is another striking example of the extreme specificity of these strains for certain tissues. This narrow specificity for certain tissues is in keeping with the fact that myositis occurs during certain epidemics of respiratory infections, and with the striking example of elective localization in a wide-spread epidemic of myositis reported by Curschmann,[20] in which the muscles of the neck were chiefly involved.

The streptococcus from myositis did not differ greatly in morphology, cultural character, and staining reactions from those isolated in other diseases studied, and the reasons for the myositis strains possessing affinity for muscles, and the other strains for the respective other tissues or organs are still obscure. The fact, however, that the killed streptococci from myositis localized in muscles, as did the live organism, indicates that the property (chemical structure, electrical charge, perhaps) on which this depends resides within the bacterial cell. The observation that the dead streptococci revealed evidence of disintegration as lesions in the muscles developed suggests that the poisons which cause the symptoms and lesions in myositis are either preformed in the bacterial cells or are formed as they disintegrate. The latter view is strongly supported by the experiments performed by one of us[21] some years ago, in which it was shown that when streptococci and other bacteria undergo digestion highly poisonous substances of a similar nature, regardless of the species, are formed during the early stages of proteolysis and that these disappear as proteolysis proceeds to a further point. According to the results of these experiments and those of Zinsser[22] and his coworkers, in which toxic substances are formed similar in their effects, irrespective of the species of micro-organism, the localizing power of bacteria rather than specific poisons becomes a factor of first importance in the etiology of myositis, as indeed it may be of other diseases.

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20. Curschmann, H.: Ueber eine Epidemie von myositischer Pseudogenickstarre, München. med. Wchnschr. **44**:1, 1917; Abstr. J. A. M. A. **68**:943, 1917.

21. Rosenow, E. C.: On the Production of Anaphylatoxic Substances by Autolysis of Bacteria and Their Relation to Endotoxins, J. Infect. Dis. **10**:113, 1912.

22. Zinsser, H., Parker, J. T., and Kuttner, A.: On Certain Poisonous Substances Produced in Bacterial Cultures, Proc. Soc. Exper. Biol. and Med. **18**:49, 1920.

The retention of the peculiar affinity during the killing process of these streptococci is in keeping with the fact discovered by Marchand,[23] that opposition of virulent organisms to phagacytosis is not destroyed by heat, and to the observation by one of us [24] that the resistance to phagocytosis of virulent pneumococci is due to an extractable substance, "virulin." The fact that the streptococci having affinity for the muscles in these cases are culturally dissimilar does not argue against their having etiologic relationship. The symptoms and the course of the disease are so variable as to suggest their not being due to a specific microorganism. The streptococci from the joints of patients with acute rheumatic fever having elective affinity, it will be recalled, were also dissimilar.[8]

The finding of the *Staphylococcus aureus* in the tonsils in Case 536 having elective affinity for muscles indicates that this organism may be the cause of mild myositis, just as Martinotti,[25] and Schmitz [26] have shown this to be so in one case each of acute polymyositis, in which this organism was isolated from the muscle lesion and was found to have elective affinity for the muscles of animals, producing lesions in muscles almost to the exclusion of lesions in other organs.

From the microscopic study of sections of muscles in chronic myositis and arthritis deformans, and of those in animals following repeated injections of these strains, a most interesting mechanism in the production of the lesions has been discovered. Evidence has been obtained as to why cure in these chronic affections is so difficult, and the reason massage and applications of heat are such valuable agents in their treatment. The reaction in these very chronic conditions is not leukocytic, but mainly mononuclear and endothelial. The endothelial cells lining the small blood vessels become extremely swollen, proliferate, and, in consequence, the lumen of vessels, including arterioles, becomes partially or completely obstructed. The supply of available oxygen is thus lowered and the growth of the organisms favored, for it has been found that the streptococci from the lesions in muscles are very sensitive to oxygen. The common occurrence of spontaneous and experimental lesions in flat muscles or in the tendinous ends where the normal blood supply, and, consequently, the supply of available oxygen in response to insult are relatively low would seem to be due to the same cause.

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23. Marchand, L.: Etude sur la phagocytose des streptocoques atténués et virulents, Arch. de méd. expér. **10**:253, 1898.

24. Rosenow, E. C.: Human Pneumococcal Opsonin and the Anti-Opsonic Substance in Virulent Pneumococci, J. Infect. Dis. **4**:285, 1907.

25. Martinotti, C.: Ueber Polymyositis acuta verursacht durch einen Staphylococcus, Centrabl. f. Bakteriol. **23**:877, 1898.

26. Schmitz, H.: Bakteriologische Untersuchung eines Falles von Polymyositis acuta, Centralbl. f. Bakteriol., Orig. **65**:259, 1912.

Our success in isolating the strains and in reproducing the lesions is due, we feel, in large part to the use of culture mediums (tall columns of glucose-brain broth or ascites broth) which afford a gradient of oxygen pressure, to the injection of animals as soon as abundant growth occurred, usually within twenty-four hours, and to the painstaking effort made to obtain bacteria from the depths of foci of infection.

Bacteria having specific affinity for muscles have been demonstrated regularly in the foci of infection and, in some instances, on the free surface of mucous membrane and in excised muscles in cases of myositis in man. With these organisms the disease has been reproduced, the organisms isolated from the experimental lesions, and demonstrated in them, and myositis again produced on reinjection. The lesions in animals, in general, corresponded in their severity and distribution to those present in the patient from whom the strains were isolated, and in that they were usually nonsuppurative in character. The number of lesions was often in proportion to the size of the dose. The conclusion, therefore, may be drawn that myositis, including even the mild transient affections of muscles, is caused in the main by lodgment and growth of bacteria, usually streptococci, which have elective affinity for muscle tissue.[27]

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27. Evans, J. S.: Focal Infections. In: Tice, F.: Practice of Medicine, Hagerstown, Md., W. F. Prior Co., 1920, **1**:

ON THE CAUSES OF THE VARIATIONS IN THE SEDIMENTATION OF THE CORPUSCLES AND THE FORMATION OF THE CRUSTA PHLOGISTICA ("SIZE," "BUFFY COAT") ON THE BLOOD

A PRELIMINARY COMMUNICATION *

H. C. GRAM

COPENHAGEN, DENMARK

If venous blood from a normal man or woman is left to clot it will, as a rule, form a homogenous red mass at the end of the coagulation.

In certain pathologic and physiologic states, the coagulum is differentiated into a lower red and an upper, more or less broad, yellowish layer (crusta phlogistica, buffy coat, size), which consists of coagulated plasma.

While this phenomenon is normal in the blood of several animals, it has been known from the earliest times that a crusta phlogistica in human blood is a pathologic phenomenon.

The newer medicine, however, generally has disregarded this symptom, possibly because it may be found in so many different states, which did not seem to form a unity. This opinion, for instance, is thus expressed by Panum [1] (1851): "Utrum crusta in sanguine appareat, necne, ex rationibus compluribus vere physicis pendet, et vix unquam licet inde certam sanguinis compositionem suspicari."

Investigations by Hewson [2] (1771) and Hunter [3] (1794) had proved that a buffy coat on the blood appeared when the sedimentation of the corpuscles or the time of coagulation was increased. Both authors put stress on the changes of the clotting time.

A crusta phlogistica was found especially in cases of inflammation, so that Borsieri [4] and Tommasini,[5] who found this phenomenon in chlorosis, thought that this disease might be characterized as an inflammation.

Panum,[1] however, warned against repeated venesection in patients because their blood showed a crusta phlogistica, since this might be caused by the bloodletting itself.

Andral [6] (1845) studied the conditions under which the crusta (couenne) formation took place clinically, and came to the conclusion

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* From the Clinic of Professor Faber, Rigshospital.

1. Panum: Dissertation (Copenhagen), 1851.
2. Hewson: Experimental Inquiries in the Properties of Blood, London, 1771.
3. Hunter: Treatise on the Blood, London, 1794.
4. Borsieri: Loco incer'o, quoted by Andral.[6]
5. Tommasini: Sull' infiammazione, quoted by Andral.[6]
6. Andral: Essai d'hématologie pathologique, Paris, 1845.

that a crusta on the blood appeared only when the fibrin percentage of the blood was increased or the number of corpuscles was decreased.

Henle,[7] Dogiel,[8] Panum,[1] Hayem,[9] Valentin[10] and Pfeiffer[11] all found that the sedimentation of the corpuscles was of extreme importance for the formation of the buffy coat, and that an increased sedimentation of the corpuscles generally was caused by an agglutination of the corpuscles.

Pfeiffer thought that an increased fibrinogen percentage might make the corpuscles sticky and thus further the agglutination.

Hekma[12] showed that the corpuscles of the horse sank more rapidly in citrated blood than in defibrinated blood, and de Haan[13] found that the velocity of sedimentation was proportional with the degree of agglutination. The cause of the agglutination according to de Haan lies in the corpuscles, though he admitted that the medium of suspension had some influence.

Kozawa[14] and Høber[15] showed that there was a parallelism between the negative electrical charge of the corpuscles in different animals and the differences between the rapidity of sedimentation found by Berkzeller and Stanker.[16]

Schwyzer[17] has investigated these relations more fully and finds that the suspension stability of the corpuscles depends on the negative charge of the corpuscles.

When this decreases, the corpuscles are attracted to one another and agglutinate. While citrate did not hamper the agglutination, a surplus of sodium chlorid (11 *pro mille*) prevented it.

Fåhraeus[18] specially studied the augmented sedimentation found during normal pregnancy. He found a proportionality between the sedimentation velocity and the degree of auto-agglutination.

The phenomenon, however, was not specific, since it was found also in most infectious diseases and in cases of cancer. Also the sedimentation was quicker in the blood of normal women than in that of normal men.

In a later paper Fåhraeus[19] discusses the causes of the changes in the stability of the blood, accepting the theory of the lowered electrical

7. Henle: Loco incerto.
8. Dogiel: Arch. f. Anat. u. Physiol., 1883, quoted by Pfeiffer.
9. Hayem: Du sang, etc., Paris.
10. Valentin: Pathologie des Blutes, Leipzig, 1866.
11. Pfeiffer: Ztschr. f. klin. Med. **33**:215, 1897.
12. Hekma: Biochem. Ztschr., Festband, p. 177, 1908.
13. de Haan: Biochem. Ztschr. **86**:298, 1918.
14. Kozawa: Biochem. Ztschr. **60**:146, 1914.
15. Høber: Arch. f. d. ges. Physiol. **101**:627, 1904.
16. Berkzeller and Stanker: Internat. Ztschr. f. physiol. chem. Biol. **3**:133, 1917.
17. Schwyzer: Biochem. Ztschr. **60**:297, 1914.
18. Fåhræus: Hygiea, 1918.
19. Fåhræus: Biochem. Ztschr. **89**:115, 1918.

charge as the reason for the augmented agglutination. He finds that the properties of the corpuscles are altered, but that the primary reason for this change is to be sought in the plasma.

As to the provocative cause, Fåhraeus remarks that the sedimentation is increased in all cases in which Abderhalden supposes that proteids foreign to the blood are introduced into the circulation.

Quite recently Westergren [20] published a paper dealing with the rapid sedimentation found in cases of pulmonary tuberculosis. In this disease he constantly finds an increased sedimentation, beyond the normal values, when citrated blood is kept in narrow tubes and observed after one hour.

In this paper there is mentioned an as yet unpublished research by Fåhraeus on the causes of the sedimentation.

While working on the causes of hemorrhagic diathesis, I have elaborated a technic,[21] by which the fibrin percentage in blood and plasma may be determined by a gravimetric method on 2 c.c. cell-free citrated plasma obtained from 5 c.c. citrated blood.

The blood (4.5 c.c.) was taken into a graduated centrifuge tube (Oluf Thomsen) containing 0.5 c.c. of a 3 per cent. citrate solution. In many cases the platelet count (Thomsen,[22] Gram [23]) was determined by Thomsen's method and also the coagulation time of citrated plasma on recalcination (Gram [24]). The tubes used were divided into 0.1 c.c., and the size chosen was such that 0.1 c.c. on the gradations equals 0.1 cm.

In order to calculate the platelet count and fibrin percentages, the cell volume was determined in all cases.

I soon found that there occurred a more or less pronounced sedimentation in these blood specimens, and that the sedimentation depended on (a) the cell volume percentage, and (b) the fibrin (fibrinogen) percentage in the plasma.

The sedimentation ordinarily was measured by observing the height in centimeters of the plasma layer formed, when the 5 c.c. citrated blood was left to stand at room temperature (about 19 C.) for ten minutes. The plasma layer was measured between the lower meniscus of the plasma surface and the red cell layer. Excepting in anemias, this borderline was nearly always quite clear and sharp as if cut with a knife.

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20. Westergren: Acta med. scand. **54**:247, 1921.
21. The technic for the fibrin determinations will be published later, together with the results in each separate case.
22. Thomsen: Acta med. scand. **53**:507, 1920.
23. Gram: Arch. Int. Med. **25**:325 (March) 1920; Acta med. scand. **54**:1, 1920.
24. Gram: Bull. Johns Hopkins Hosp. **31**:364, 1920 (see corrections in the following issues).

In some ways it is most convenient to use an observation time of only ten minutes, but one must remember that cases with very different, generally normal fibrin percentages and cell volumes are thus included in the same group showing less than 0.1 cm. (i. e. traces) of sedimentation. However, in many pathologic cases the sedimentation after one hour has progressed as far as it will go, thus making the differences in these cases smaller than by an observation after ten minutes.

The formation of the crusta phlogistica was observed by letting 1 cm.³ of blood flow down in a miniature test tube having a diameter of from 9 to 10 mm., leaving it to clot in an upright position of the glass. The reaction could not be measured exactly, since the border between crusta and corpuscles generally is not very sharp. I have distinguished between: — (no crusta), traces, + (positive) and ++ (very broad).

The result of the cell volume determinations has been, that the cell volume in normal women oscillates about an average of 41 per cent. of the pure blood and between the limits 37 and 45 per cent.

In normal men, the cell volume oscillates about 48 per cent., and between the extremes 43 and 51 per cent.

A low cell volume, of course, was found in simple anemias, pernicious anemia and most cases of leukemia, pseudoleukemia and several other diseases of the blood. Some infectious diseases, as, for instance, rheumatic fever, typhoid, erysipelas and chronic tuberculosis, very often showed a low cell volume.

In cases of nephritis, and in cases of polyarthritis chronica and malignant tumors one may find a low cell volume. The same is true in normal pregnancy, which, especially in the earlier months, shows a distinct drop in the cell volume percentages.

The fibrin percentage per 100 cm.³ plasma—which is the value to be considered—in twenty-five normal individuals of each sex varied between 0.20 and 0.38 per cent., the mean values being 0.27 per cent. in men and 0.29 per cent. in women, that is, slightly lower in men than in women. These different levels of fibrin content are held from one time to another by the same normal individual.

In many chronic noninfectious diseases no deviations were found from the normal fibrin content of the plasma and the normal cell volume.

In the description of each separate disease, the number of cases observed and the average and variations of the fibrin percentage in plasma is added in brackets.

A fibrin percentage in plasma below the lowest normal limit was met with in some but not nearly in all cases of severe degeneration of the liver.

Out of fourteen such cases, six showed a fibrin percentage in plasma smaller than 0.20 per cent., and the average was 0.22 per cent. (variations from 0.39 to 0.07 per cent.). In some of these cases, when infectious complications were present, it appears that the normal fibrin percentage found must be considered pathologic. The diagnosis in all cases was verified postmortem.

Outside this group a fibrin deficiency was found only in a few cases of pernicious anemia [25] (fatty degeneration of the liver?) and one case of profuse carcinomatosis of the liver.

In simple anemia (fifteen cases, average 0.33 per cent., variations from 0.40 to 0.25 per cent.) the fibrin percentage in plasma was normal, though the percentage per 100 c.c. blood was very high.

In polycythemia (eight cases, average 0.30 per cent., variations from 0.40 to 0.21 per cent.) the percentage in plasma also varied nearly within the normal limits, while the percentage per 100 c.c. blood was very low. This means that the fibrin percentage in plasma, not that in blood, is the value which remains fairly constant, when the cell volume is altered.

In leukemia (fourteen cases, average 0.37 per cent., variations from 0.46 to 0.29 per cent.), especially in the myeloid type, one may find a slight absolute increase of the fibrin percentage in the plasma. The same is true in hemophilia (three cases, average 0.40 per cent., variations from 0.43 to 0.37 per cent.).

One case of purpura and one of pseudoleukemia showed normal fibrin percentages (respectively 0.31 and 0.34 per cent.), while a case of scurvy with gingivitis and loosened teeth had a slight increase (0.41 per cent.).

The increase is very pronounced and always absolute (i. e., > 0.38 per cent.) in lobar pneumonia (seven cases, average 1.05 per cent., variations from 1.50 to 0.80 per cent), bronchopneumonia (five cases, average 0.75 per cent., variations from 1.29 to 0.52 per cent), rheumatic fever (nine cases, average 0.77 per cent., variations from 0.94 to 0.55 per cent.), pleurisy (eight cases, average 0.74 per cent., variations from 1.07 to 0.55 per cent.), erysipelas (four cases, average 0.72 per cent., variations from 0.87 to 0.58 per cent.), suppurative infections (sixteen cases, average 0.68 per cent., variations from 1.01 to 0.46 per cent.), diphtheria (five cases, average 0.63 per cent., variations from 0.73 to 0.48 per cent.), gonorrheal "metastasis" (four cases, average 0.61 per cent., variations from 0.81 to 0.48 per cent.), scarlet fever (sixteen cases, average 0.60 per cent., variations from 0.83 to 0.45 per cent.), angina (ten cases, average 0.57 per cent., variations from 0.80

25. Pernicious anemia: 23 cases, average 0.26 per cent., variations from 0.37 to 0.10 per cent. Only four cases show less than 0.20 per cent. fibrin in the plasma.

to 0.46 per cent.), and bronchitis (fifteen cases, average 0.57 per cent., variations from 0.91 to 0.40 per cent.).

The increase is slighter and may keep within the upper boundaries of the normal in uncomplicated measles (two cases, average 0.42 per cent., variations from 0.42 to 0.43 per cent), typhoid (five cases, average 0.44 per cent., variations from 0.50 to 0.36 per cent.), influenza (thirty-three cases, average 0.38 per cent., variations from 0.48 to 0.30 per cent.), "closed tuberculosis" (seven cases, average 0.36 per cent., variations from 0.43 to 0.25 per cent.), malaria (three cases, average 0.35 per cent., variations from 0.38 to 0.30 per cent.), and syphilis (six fresh cases, average 0.35 per cent., variations from 0.42 to 0.27 per cent. and six old cases, average 0.47 per cent., variations from 0.66 to 0.31 per cent).

If one of these last named infections is complicated by one belonging to the first group, there occurs an immediate rise in the fibrin percentage.

This was demonstrated in: (1) measles with bronchitis or otitis (four cases, average 0.53 per cent., variations from 0.58 to 0.47 per cent.); (2) influenza with bronchitis (five cases, average 0.55 per cent., variations from 0.66 to 0.48 per cent.); (3) influenza with angina or otitis (three cases, average 0.58 per cent., variations from 0.60 to 0.57 per cent.); (4) influenzal pneumonia (twenty-two cases, average 0.62 per cent., variations from 0.98 to 0.36 per cent.); (5) tuberculosis of the lungs (twenty-one cases, average 0.62 per cent., variations from 1.22 to 0.33 per cent.).

Outside of the infectious diseases proper, an increased fibrin percentage in the plasma was often or always found in the following pathologic and physiologic states: (1) After intramuscular injection of sterile milk (two cases, average 0.465 per cent., variations from 0.48 to 0.45 per cent.); (2) polyarthritis chronica progressiva (ten cases, average 0.525 per cent., variations from 0.70 to 0.36 per cent.); (3) malignant tumors (excluding cancer of the liver or pancreas: twenty-six cases, average 0.55 per cent., variations from 0.81 to 0.31 per cent.); (4) nephritis (thirteen uncomplicated cases, average 0.41 per cent., variations from 0.80 to 0.27 per cent.; ten cases with fever or lung complications, average 0.62 per cent., variations from 0.89 to 0.50 per cent.); (5) pregnancy (second month, one case, average 0.33 per cent.; third month, one case, average 0.42 per cent.; fourth month, three cases, average 0.36 per cent., variations from 0.39 to 0.33 per cent.; fifth month, four cases, average 0.39 per cent., variations from 0.44 to 0.32 per cent.; sixth month, nine cases, average 0.43 per cent., variations from 0.49 to 0.32 per cent.; seventh month, ten cases, average 0.46 per cent., variations from 0.52 to 0.40 per cent.; eighth month, seven cases, average 0.57 per cent., variations from 0.83 to 0.44 per cent.; ninth

TABLE 1.—Sedimentation After Ten Minutes (Abscissa), Cell-Volume Percentage (Ordinate) and Fibrin Percentage in Plasma (Rubric Numerals) in Five Hundred and Forty-Two Blood Specimens. The Rubric Numerals Are Mean Values of the Specimens, Which Show an Equal Sedimentation and a Nearly Equal Cell-Volume*

	Sedimentation after 10 Minutes in Centimeters																										
	0	Trace	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6
0-10	....	....	....	0.10	....	....	....	....	....	....	....	0.33	....	....	....	....	....	....	....	....	....	0.42						
11-15	....	....	....	....	0.26	0.32	0.32	....	....	0.39	0.32	0.27	....	0.44														
16-20	....	....	....	0.24	0.23	0.26	0.28	0.31	....	0.85	....	....	....	0.30	....	....	....	....	....	....	....	....	....	....	....	....	....	0.82
21-25	....	0.20	0.25	0.28	0.30	0.31	0.40	0.38	....	0.46	....	....	0.58	....	....	....	0.61	0.59	0.63									
26-30	....	0.10	0.27	0.24	0.27	0.32	0.37	0.43	0.48	....	0.33	....	....	0.72	....	0.72	0.69	....	....	....	0.78	....	....	....	....	....	0.92	
31-35	....	....	0.29	0.38	0.47	0.52	0.65	....	0.56	....	0.71	....	0.94	....	....	0.70	0.78	0.94										
36-40	....	0.33	0.47	0.51	0.53	0.59	0.58	0.79	0.78	1.02	0.62	....	0.75	0.71	....	....	0.85	0.83	....	....	....	....	....	0.98				
41-45	....	0.34	0.52	0.59	0.58	0.55	0.51	0.70	0.86	....	....	....	....	....	0.97	1.22												
46-50	0.24	0.35	0.49	....	....	....	0.73																					
51-55	0.25	0.34																										
56-60																												
61-65	0.28																											
66-70	0.30																											

* In considering this table one must remember that a sedimentation <0.1 cm. after ten minutes may be met with in specimens with a normal cell volume, but a slightly increased fibrin percentage. In severe anemia the borderline between plasma and corpuscles is not clearly defined, so that the reading must be somewhat arbitrary.

month, seven cases, average 0.49 per cent., variations from 0.56 to 0.45 per cent.; tenth month, eighteen cases, average 0.55 per cent., variations from 0.74 to 0.39 per cent); (6) catarrhal jaundice (four cases, average 0.38 per cent., variations from 0.54 to 0.29 per cent.); (7) cholelithiasis (two cases, average 0.415 per cent., variations from 0.42 to 0.41 per cent.).

These "hyperinoses" may in some cases be explained by complicating infections.

This holds true in connection with the increased fibrin percentages found in many cases of decompensated heart disease and some cases of nephritis with stasis or edema of the lungs. Sometimes, however, the stasis in the liver counteracts the influence of the infection in the lungs, making the fibrin percentage normal or even decreased.

TABLE 2.—INFLUENCE OF VARIATIONS IN FIBRIN PERCENTAGE AND CELL VOLUME

Diagnosis	Date	Sedimentation in 10 Minutes, Cm.	Crusta Phlogistica	Fibrin in Plasma, per Cent.	Cell-Volume, per Cent.
1. Simple anemia	1/ 6	0.2	+	0.32	21
	1/17	0.2	Traces	0.29	21
	2/10	0.1	+	0.32	21
	2/24	0.1	+	0.29	25.5
	3/ 9	0.1	—	0.26	33
	3/23	Traces	—	0.30	39
	3/30	Traces	—	0.31	41
2. Rheumatic fever, anemia	2/ 1	1.6	++	0.94	34
	2/14	0.3	+	0.45	34
	2/21	0.2	+	0.43	37
	2/28	Traces	—	0.35	35.5
	3/ 6	0.1	—	0.32	38

The hyperinosis found in cases of malignant tumors may partly be explained by infection of ulcerated surfaces, and, in fact, one case with a closed, small cancer in the vertebral column showed no absolute increase. Still, there can be no doubt that the tumor itself exerts some influence in the same direction.

Icterus per stasis in cases of cancer of the pancreas does not prevent the hyperinosis (four cases, average 0.63 per cent., variations from 0.97 to 0.50 per cent.). In secondary, diffuse cancer of the liver the fibrin percentage in plasma may rather often, especially in the last stages, be found normal or even decreased (seven cases, average 0.47 per cent., variations from 0.72 to 0.27 per cent. [first examinations!]).

From the beginning of normal pregnancy [26] there occurs a tendency to hyperinosis, which however does not always pass beyond the upper boundary of normal before in the fifth or sixth month.

26. At the same time there is a more or less pronounced tendency to anemia.

There is no special increase in cases of eclampsia, in which, however, the cell volume is markedly higher than in normal pregnant women (three cases, average 0.57 per cent., variations from 0.70 to 0.48 per cent.).

The sedimentation of the corpuscles was studied on 542 specimens of citrated blood.

The result of this series (Table 1) was that a sedimentation of 0.1 cm. or more after ten minutes occurred only: (1) when the fibrin percentage in plasma was higher than normal, or (2) when the cell volume was lower than normal.

The effect of variations in these factors is shown in Table 2.

In order to demonstrate the effects of the finer changes in the fibrin percentage, a longer time of observation is necessary, but this has only been done in a few cases, which, however, showed that the sedimentation in normal blood was regulated by the same laws.

TABLE 3.—CELL VOLUME, FIBRIN PERCENTAGE AND SEDIMENTATION AFTER ONE HOUR IN TEN INDIVIDUALS OF EACH SEX

Number	Cell-Volume, per Cent.	Fibrin Percentage in Plasma	Sedimentation after 1 Hour	Number	Cell-Volume, per Cent.	Fibrin Percentage in Plasma	Sedimentation after 1 Hour
1	38	0.37	1.3	1	49	0.37	0.5
2	40	0.33	0.8	2	49	0.35	0.4
3	38	0.29	0.6	3	46	0.33	0.3
4	43	0.31	0.6	4	49	0.31	0.1
5	45	0.35	0.5	5	46	0.24	0.1
6	40	0.26	0.5	6	49	0.27	<0.1
7	40	0.27	0.4	7	48	0.24	<0.1
8	42	0.24	0.4	8	45	0.21	<0.1
9	43	0.27	0.4	9	46	0.225	<0.1
10	46	0.32	0.2	10	49	0.23	<0.1
Average	41.5	0.30	0.6	..	48	0.27	<0.2

After ten minutes one may observe, that the plasma layer formed is broader in women's than in men's blood, where it may be missing altogether.

An observation of citrated blood from ten normal women and the same number of men after one hour has given the results shown in Table 3. Women to the left, men to the right side.

From Table 3 it appears that the difference between the sedimentation in the blood of men and women is very pronounced, and that the difference in fibrin percentage will only partly explain this.

The question now is, whether the difference in the average cell volume can explain the different sedimentation found in the two sexes.

To decide this I made the following experiments: 20 cm.^{3} of citrated blood is taken from a normal woman, and three Oluf Thomsen tubes are each filled with exactly 5 c.c. of this.

The two specimens (1 and 2) are left to stand for one hour, and the sedimentation in centimeters is noted.

The third specimen (3) is centrifuged for one and one-half hours. The cell volume and the fibrin percentage in plasma is determined.

To Specimen 2 a little of the precipitate from Specimen 3 is added and a corresponding quantity of the plasma is taken off.

Specimens 1 and 2b are then shaken energetically, and left to stand for another hour, the sedimentation again being noted in both.

Both specimens (1 and 2b) are then centrifuged and the cell volume in both is determined.

TABLE 4.—Sedimentation After One Hour in Citrated Blood Before and After an Artificial Increase of the Cell Volume: Two Cases

	Sedimentation after 1 Hour	Cell Volume, per Cent.	Fibrin Percentage in Plasma
Experiment A:			
1	0.9	40	0.34
2	0.9	40	0.34
1b	0.8	40	0.34
2b	0.3	47	0.34
Experiment B:			
1	0.4	43	0.27
2	0.4	42	0.27
1b	0.35	42	0.27
2b	<0.1	49	0.27

These two experiments (Table 4) seem to show that the principal cause of the difference in sedimentation between the blood of the sexes must be sought in the different averages of the cell volume.

There can be no doubt that there is a considerable experimental error in the determination of the velocity of sedimentation by this method. However, a series of double specimens and double specimens with variations in the amount of blood added to the citrate gave nearly uniform values.

A more serious error is introduced by the variations in the room temperature. This is shown in Table 5 on two double specimens which are kept respectively at 20 and 28 Celsius.

TABLE 5.—Sedimentation in Two Pathologic Double Specimens Kept at Different Temperatures *

Sedimentation in Specimen I Kept at 20 Celsius, Cm.	Sedimentation in Specimen II Kept at 28 Celsius, Cm.
1.5	2.0
0.3	0.5

* The sedimentation increases with the surrounding temperature till 30 Celsius.

It may also be questioned whether it is the number of cells or the cell volume which influences the velocity of sedimentation.

This was decided by determining the sedimentation on two specimens, respectively, from a case of simple and a case of pernicious anemia (Table 6) with the same cell volume and nearly the same fibrin percentage.

In order to demonstrate more directly that the sedimentation depends on the cell volume and the fibrin (fibrinogen) percentage in plasma, I have carried out a series of special experiments.

The sedimentation was measured in the same (Oluf Thomsen) glasses and in the same way as previously.

In these experiments measured quantities of corpuscles were suspended in various organic and inorganic liquids.

These corpuscles and liquids were prepared in the following way:

(1) *Washed Corpuscles.*—From 5 to 25 cm. of blood is defibrinated, the corpuscles are centrifuged down and the serum is pipetted off. The corpuscles then are washed in 0.9 per cent. sodium chlorid and thrown down several times. The last centrifuging is specially energetic. The corpuscular mass is made homogenous by shaking.

(2) *Citrated Serum.*—Nine parts of venous blood is defibrinated. One then adds one part of a 3 per cent. citrate solution, centrifuges and removes the citrated serum.

(3) *Citrated Plasma.*—This is prepared by centrifugalization for one and one-half hours of nine parts of blood + one part of a 3 per cent. citrate solution. All cellular elements are thrown down.

TABLE 6.—The Sedimentation Depending on the Cell Volume

Diagnosis	Erythrocytes, Millions per MM.	Fibrin Percentage in Plasma	Cell Volume per Cent.	Sedimentation in 10 Minutes
Anemia simplex.....	3.66	0.30	24	0.3
Anemia perniciosa.....	1.73	0.29	24	0.4

(4) *Fibrinogen-Free Plasma.*—This is prepared by heating citrated plasma to 60 C. in ten minutes. The precipitated fibrinogen is thrown down by centrifugalization.

(5) *Pure Fibrinogen.*[27]—This is prepared by mixing equal volumes of citrated plasma and saturated sodium chlorid solution. The precipitated fibrinogen is redissolved in 0.9 per cent. sodium chlorid. The process is repeated three or four times. The fibrinogen content of the final solution is determined by adding fresh serum to a few c.c. The solution is dialyzed over night against 0.9 per cent. sodium chlorid to free it of its surplus of chlorids which prevent the agglutination.

(6) *Physiological Sodium Chlorid Solution.*—0.9 per cent. sodium chlorid.

The questions to be solved by this series of experiments are the following:

(1) *Is the rapid sedimentation in pathologic blood specimen due to properties of the corpuscles or the plasma?*

27. The process is rather delicate and possibly newer methods may give better results (Johns Hopkins Hosp. Bull., 1921, loco incerto).

The experiment shown in Table 7 seems to show that the influence of the plasma is prevalent, though the sedimentation is slower and only speeds up later, when normal corpuscles are suspended in pathologic plasma.

TABLE 7.—Washed Corpuscles from a Patient with Tuberculosis Pulmonum (Fibrin Percentage in Plasma 0.59) and from a Normal Individual. Citrated Plasma from the Same Persons

Time After Shaking	1.5 Cm.³ Tuberculosis Corpuscles + 3.5 Cm.³ Tuberculosis Plasma	1.5 Cm.³ Normal Corpuscles + 3.5 Cm.³ Tuberculosis Plasma	1.5 Cm.³ Tuberculosis Corpuscles + 3.5 Cm.³ Normal Plasma	1.5 Cm.³ Normal Corpuscles + 3.5 Cm.³ Normal Plasma
	Sedimentation	Sedimentation	Sedimentation	Sedimentation
5 min.	0.2	Traces	Traces	Traces
10 min.	0.8	0.1	0.1	0.1
15 min.	1.7	0.2	0.2	0.3
20 min.	2.3	1.4	0.4	0.4
25 min.	2.8	2.3	0.5	0.6
30 min.	3.0	2.6	0.7	0.8

On the other hand, there is no considerable difference in sedimentation when respectively "normal" and "pathologic" corpuscles are suspended in normal plasma.

(2) *Is the velocity of sedimentation altered when the corpuscles are suspended in citrated serum instead of citrated plasma from the same individual?*

The experiment described in Table 8 shows that the sedimentation is enormously decreased when citrated serum is used instead of citrated plasma.[28]

TABLE 8.—Washed Corpuscles from a Patient with Rheumatic Fever (Fibrin Percentage in Plasma 0.90), Citrated Plasma and Citrated Serum from the Same Patient

Time After Shaking	2 Cm.³ Corpuscles + 3 Cm.³ Citrated Serum	2 Cm.³ Corpuscles + 3 Cm.³ Citrated Plasma	1 Cm.³ Corpuscles + 4 Cm.³ Citrated Serum	1 Cm.³ Corpuscles + 4 Cm.³ Citrated Plasma
	Sedimentation	Sedimentation	Sedimentation	Sedimentation
10 min.	0	0.2	0.2	2.0
15 min.	Traces	0.8	0.3	3.2
20 min.	Traces	1.4	0.6	3.5
25 min.	0.1	1.9	0.9	3.6
30 min.	0.1	2.2	1.2	3.7

The experiment is carried out twice, respectively with 1 and 2 cm.³ corpuscles. It also shows that a lowering of the cell volume increases the sedimentation both in plasma and in serum.

28. As the platelets and all other cells are thrown down by the preparation of the plasma, their absence in the serum cannot be the cause of the difference between these two mediums of suspension.

(3) *Is the sedimentation altered, when the corpuscles are suspended in "fibrinogen free" plasma instead of citrated plasma from the same individual?*

The experiment shown in Table 9 shows that the sedimentation, ceteris paribus, is decreased considerably when the corpuscles are suspended in a plasma deprived of its fibrinogen by heat coagulation.

TABLE 9.—Washed Corpuscles from a Patient Suffering from Nephritis (Fibrin Percentage in Plasma 0.48), Citrated Plasma and "Fibrinogen-Free" Plasma from the Same Patient

Time After Shaking	2 Cm.³ Corpuscles + 3 Cm.³ Fibrinogen-free Plasma	2 Cm.³ Corpuscles + 3 Cm.³ Citrated Plasma	1 Cm.³ Corpuscles + 4 Cm.³ Fibrinogen-free Plasma	1 Cm.³ Corpuscles + 4 Cm.³ Citrated Plasma
	Sedimentation	Sedimentation	Sedimentation	Sedimentation
10 min.	Traces	0.1	0.5	1.0
15 min.	0.1	0.3	0.9	1.7
20 min.	0.2	0.5	1.3	2.4
25 min.	0.2	0.8	1.7	3.0
30 min.	0.3	1.0	1.9	3.2

As in the previous experiment, this also shows that a lowering of the cell volume increases the sedimentation both in ordinary and in fibrinogen free plasma.

(4) *Is the sedimentation different when corpuscles are suspended respectively in physiologic sodium chlorid solution and in citrated serum?*

TABLE 10.—Washed Corpuscles and Citrated Serum from the Same Person with Presumably Normal Fibrin Percentage. Physiologic Sodium Chlorid Solution *

Time After Shaking	2 Cm.³ Corpuscles + 3 Cm.³ 0.9% NaCl	2 Cm.³ Corpuscles + 3 Cm.³ Citrated Serum	1 Cm.³ Corpuscles + 4 Cm.³ 0.9% NaCl	1 Cm.³ Corpuscles + 4 Cm.³ Citrated Serum
	Sedimentation	Sedimentation	Sedimentation	Sedimentation
10 min.	Traces	Traces	Traces	<0.1
15 min.	Traces	<0.1	<0.1	0.1
20 min.	Traces	<0.1	<0.1	0.1
25 min.	Traces	<0.1	<0.1	0.2
30 min.	Traces	<.01	<0.1	0.3
24 hrs.	0.5	1.9	2.8	3.6

* In this and the following tables I have distinguished between traces of sedimentation and a sedimentation <0.1, which latter means that the lower meniscus of the plasma (serum) surface is visible.

The experiment shown in Table 10 shows that the sedimentation, ceteris paribus, is more rapid in citrated serum than in physiologic sodium chlorid solution. This, however, may partly be due to the action of the sodium chlorid. As in the previous experiments, this has been done with different cell volumes, which shows that a lowering of the cell volume increases the sedimentation both in citrated serum and in physiologic sodium chlorid solution.

The specimens have been observed for a longer period, since the sedimentation is very slow.

(5) *Do the corpuscles settle more quickly in pathologic than in normal citrated serum?*

The experiment shown in Table 11 tends to show that the corpuscles settle a little more quickly in citrated serum from cases with hyperinosis than serum from normal individuals.

(6) *Will the addition of fibrinogen to a liquid cause a more rapid sedimentation of the corpuscles suspended therein?*

The addition of "pure" fibrinogen to a 0.9 per cent. sodium chlorid solution at first caused a distinct decrease in the sedimentation. The fibrinogen contained a large amount of sodium chlorid, which in concentrations above 1 per cent. (compare: Schwyzer [17]) prevented the agglutination.

TABLE 11.—Washed Corpuscles from a Normal Person (Fibrin Percentage in Citrated Plasma 0.21), Citrated Serum from the Same Person and from a Patient with Pleurisy (Fibrin Percentage in Citrated Plasma 0.51)

Time After Shaking	2 Cm.³ Normal Corpuscles + 3 Cm.³ Normal Citrated Serum	2 Cm.³ Normal Corpuscles + 3 Cm.³ Pleurisy Citrated Serum
	Sedimentation	Sedimentation
10 min.	Traces	Traces
20 min.	Traces	Traces
30 min.	Traces	<0.1
40 min.	Traces	<0.1
50 min.	<0.1	<0.1
60 min.	<0.1	0.1

When the salt was eliminated by dialysis, the results as shown in Tables 12 and 13 were quite clear and showed that even a low fibrinogen content will increase the sedimentation in physiologic sodium chlorid solution.

Microscopy of citrated blood has shown that the corpuscles on standing are agglutinated and then settle more quickly. This phenomenon is more pronounced the more fibrinogen the plasma contains.

About these "auto-agglutinins," which cause the agglutination of the corpuscles, we know the following:

(1) Their action varies proportionally with the amount of fibrinogen in the plasma.

(2) Their action is extremely weakened, when the fibrinogen is eliminated by spontaneous coagulation.

(3) Their action is also weakened when the fibrinogen is eliminated by heat coagulation at 60 C.

(4) The pure fibrinogen prepared by repeated precipitation with sodium chlorid shows—when freed of sodium chlorid—the agglutinating action of these bodies.

(5) The serum shows a slight agglutinating action which is stronger in serum whose plasma shows hyperinosis.

Since we know these "agglutinins" only by their action, and this is intimately connected with the fibrinogen, one may fancy that it is the fibrinogen and in a lesser degree other plasma proteids which possess these properties.

TABLE 12.—Washed Corpuscles and Pure Fibrinogen from a Normal Individual. The Fibrinogen Is Dissolved in 0.9 Per Cent. Sodium Chlorid (0.19 Per Cent.)

Time After Shaking	1.5 Cm.³ Corpuscles + 3.5 Cm.³ 0.9% NaCl	1.5 Cm.³ Corpuscles + 3.5 Cm.³ Fibrinogen Solution
	Sedimentation	Sedimentation
5 min.	0	Traces
10 min.	Traces	<0.1
15 min.	Traces	<0.1
20 min.	Traces	<0.1
25 min.	<0.1	0.1
30 min.	<0.1	0.1

Whether the action of these bodies in the last instance depends on their influence on the electrical charge of the corpuscles is not shown by my experiments.

The results of the examination for crusta formation has shown that only in one out of twenty-five women and in no normal man were there found traces of a "buffy coat" on the blood.

The woman mentioned had a rather high normal fibrin percentage and a cell volume at the very lowest limit of the normal.

TABLE 13.—Washed Corpuscles and Fibrinogen from a Normal Individual. The Fibrinogen Is Dissolved in 0.9 Per Cent. Sodium Chlorid (0.22 Per Cent.). The Experiment Has Been Made on Double Specimens

Time After Shaking	1.5 Cm.³ Corpuscles + 3.5 Cm.³ 0.9% NaCl	1.5 Cm.³ Corpuscles + 3.5 Cm.³ 0.9% NaCl	1.5 Cm.³ Corpuscles + 3.5 Cm.³ Fibrinogen Solution	1.5 Cm.³ Corpuscles + 3.5 Cm.³ Fibrinogen Solution
	Sedimentation	Sedimentation	Sedimentation	Sedimentation
5 min.	0	0	Traces	Traces
10 min.	Traces	Traces	Traces	Traces
15 min.	Traces	Traces	<0.1	<0.1
20 min.	Traces	Traces	<0.1	<0.1
25 min.	Traces	Traces	<0.1	<0.1
30 min.	Traces	Traces	0.1	0.1
60 min.	<0.1	<0.1	0.2	0.2
Later	0.1	0.1	0.5	0.5

The clinical investigations seemed to show that a crusta phlogistica is formed under two circumstances: (1) when the sedimentation of the corpuscles is more rapid than usual, and (2) when the coagulation time is longer than usual.

As mentioned above, the velocity of sedimentation is increased when the cell volume is low (anemia) or the fibrin (fibrinogen) content of the plasma is increased (hyperinosis).

The velocity of sedimentation also is increased when the temperature of the blood is higher.

A determination of the coagulation time of recalcinated plasma (Gram [24]) has shown that the changes in this value correspond well with the coagulation time of "natural" blood. A possible exception may be found in some cases of icterus.

A lengthening of the clotting time may be found under two circumstances: (a) as a result of an alteration in the composition of the blood, and (b) as a result of the conditions under which the blood is taken and kept.

In hemophilia and in diseases which are accompanied by thrombopenia (pernicious anemia, lymphatic leukemia, purpura idiopathica and influenzal pneumonia (Gram [24])) the coagulation time may be longer than usual (i. e. > 6 minutes).

If the blood is kept in a paraffinated glass or at low temperature the clotting time is prolonged.

TABLE 14.—THE PROPORTIONAL DIVISION OF THE CASES ACCORDING TO THE SIZE OF THE CRUSTA WITHIN THE DIFFERENT DEGREES OF SEDIMENTATION. FIVE HUNDRED AND TWENTY-SIX CASES

Sedimentation in 10 Minutes Cm.	Crusta Phlogistica			
	— Per Cent.	Traces Per Cent.	+ Per Cent.	++ Per Cent.
0	100	0	0	0
Traces	80	8	2	0
0.1	25	27	58	0
0.2	3	20	77	0
0.3	0	6	91	3
0.4	0	12	83	4
0.5	0	6	76	18
0.6 and more	0	7	40	53

A consideration of my material shows with great certainty, that the first factor (sedimentation) is the most frequent and important cause for the formation of a buffy coat.

If we arrange the correlated values for sedimentation and crusta formation in classes, we get the results shown in Table 14.

Table 14 shows that in some cases no crusta is met with, though the sedimentation is clearly increased. This, however, happens only when the increase is relatively slight (from 0.1 to 0.2 cm. in ten minutes).

This may be due to circumstances connected with the taking and the conservation of the blood. A bad venepuncture or unclean glasses may shorten the coagulation time of the blood, and thus prevent the crusta formation.

Variations in the room temperature will be compensated partly, since an increase of the temperature makes the sedimentation more rapid, but on the other hand shortens the clotting time.

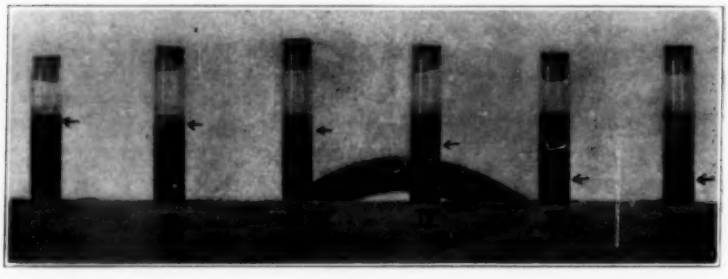
According to Table 14 one finds some cases with only traces (i. e., < 0.1 cm.) of sedimentation in ten minutes, but with a more or

less pronounced crusta. These variations may partly be explained by circumstances connected with the taking and conservation of the blood.[29]

If we consider the diagnosis in the last named cases we find, however, that nearly all of them may be placed in one of two groups: (1) Patients with a high normal or slightly increased fibrin percentage in plasma or a low normal or slightly decreased cell volume; (2) patients with thrombopenia (pupura, pernicious anemia, influenza) or hemophilia.

A crusta phlogistica in venous blood, when 1 cm.³ is kept at room temperature in a test tube with a diameter of 9 or 10 mm. is nearly always a pathologic phenomenon, which may be due to anemia, hyperinosis, thrombopenia or hemophilia.

TABLE 15.—Sedimentation After One Hour in Six Normal and Pathologic Specimens of Citrated Blood



Number (from the left)	I	II	III	IV	V*	VI
Diagnosis	Normal man	Normal man	Normal woman	Nephritis	Pernicious anemia	Pneumonia
Sedimentation after 1 hour, Cm.	<0.1	0.2	0.4	1.1	2.6	2.8
Cell-volume per Cent.	49	51	42	47	10	42
Fibrin, per Cent. in plasma	0.29	0.34	0.27	0.44	0.27	1.31

* Owing to the intense yellow color of the plasma the photograph does not show any difference between plasma and corpuscles in this case (nr. V). The border-line however was clearly visible to the naked eye and is marked by an arrow on the figure.

SUMMARY

1. In a large number (many hundreds) of cases the fibrin percentage in plasma (and blood), the cell volume percentage and sometimes the coagulation time has been determined.

29. A small series of experiments has proved that the use of a paraffinated glass may cause crusta formation in the blood of some normal women and even a few men. The best results are obtained when the needle also is paraffinated.

2. The fibrin content of the plasma is found to be increased in nearly all infectious diseases, in cancer, nephritis, pregnancy, polyarthritis and after infections of sterile milk. These alterations may all be considered as the effect of the introduction into the circulation of proteids foreign to the blood. A low fibrin percentage in plasma is only found in some, but not nearly all, severe degenerations of the liver.

3. The cell volume, besides in the diseases of the blood proper, is frequently found low, in certain infections, in cancer, pregnancy and polyarthritis.

4. In specimens of citrated blood, the sedimentation has been studied by an observation of the plasma layer formed after ten minutes. Under the conditions described, the formation of a plasma layer of 0.1 cm. or more must be considered distinctly pathologic and is found only in cases showing anemia or hyperinosis.

A clearer analysis of the influence of the fibrin and cell volume percentages may be made by observing the plasma layer formed after one hour. The difference in sedimentation as between normal men and women is shown to depend mainly on the different cell-volumes, less on differences in the fibrin percentage.

5. The formation of the crusta phlogistica (in pathologic cases) has been observed in 1 cm.^{3} of venous blood kept at room temperature in a miniature testtube. The formation of a buffy coat generally coincides with a sedimentation of 0.1 cm. or more in the citrated blood, divergences being found only in border cases and in cases with a lengthened clotting of the blood.

6. From clinical and experimental observations we reach the following conclusions: The formation of a crusta phlogistica is a pathologic phenomenon which depends on (a) an accelerated sedimentation, or (b) a lengthened clotting time.

The sedimentation of the corpuscles depends on: (1) the fibrin (fibrinogen) percentage in the plasma, the sedimentation being accelerated by a rise in this value, and vice versa. The fibrinogen brings this about by causing an agglutination of the corpuscles, which facilitates their sedimentation; (2) the cell volume percentage, the sedimentation being accelerated by a drop in this value, and vice versa; (3) the temperature, the sedimentation being accelerated when the temperature is higher, and vice versa.

The first two factors may counteract or assist one another, so that a knowledge of one of these, together with a determination of the velocity of sedimentation, allows an estimate of the other factor, that is, if the temperature of the surroundings is constant.

The coagulation time of the blood is found lengthened in hemophilia, and diseases which cause a thrombopenia, i. e., pernicious anemia, lymphatic leukemia, influenzal pneumonia, etc.

This last factor is not nearly so important, practically, as the velocity of sedimentation.

However, an accidental shortening of the clotting time may occasionally prevent the formation of a crusta, though the sedimentation is more rapid than normally.

The three factors mainly affecting the coagulation time are: (1) platelet count; (2) properties of the plasma (hemophilia), and (3) temperature.

7. A single specimen of 4.5 cm.³ blood taken into an Oluf Thomsen glass (graduated centrifuge tube) allows the determination of the following values: (1) platelet count (Oluf Thomsen [22]); (2) coagulation time (Gram [24]); (3) plasma color (Meulengracht [30]); (4) fibrin percentage in plasma and blood; (5) cell volume percentage; (6) velocity of sedimentation.

Another specimen of 1 cm.³ allows an observation of the crusta formation.

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30. Meulengracht: Ugeskr. f. Læger **46**:1785, 1919.

OBSERVATIONS ON THE RELATION OF UREA TO UREMIA *

LOUIS LEITER
CHICAGO

The objects of this research were to determine what relationship existed between the toxicity of various normal urinary constituents and the manifestations of what is clinically known as uremia—meaning by the term "uremia" the symptoms observed as a result of marked obstruction to the outflow of urine, on the one hand, and, on the other hand, the symptoms which appear in many cases of chronic Bright's disease, characterized by convulsions, various paralyses and coma.

In pursuance of these objects, it was thought advisable to begin with the simplest organic urinary constituent—urea—to determine its toxicity on dogs—when injected in large doses in relatively short periods of time—to note the symptoms produced, to follow the changes in the blood and urine content of urea, and to determine, at the necropsy, the pathologic changes that might have taken place—both gross and microscopic—as well as the urea content of various body fluids. Then, the toxicity of urea was determined in dogs in which one ureter had been ligated; and in other cases both ureters were ligated and the changes in the blood urea followed until the death of the animal.

THE LITERATURE

There is a tremendous literature [1] on the subject of uremia and related topics; but, while theories are abundant, and there are many facts, there is a great deal of conflict of opinion on every aspect of the subject—even on the subject as to what constitutes "uremia."

One of the earliest theories of the causation of uremia was a chemical theory put forth by Wilson in 1833, in which the symptoms were ascribed to the retention of urea in the blood. But discrepancies were soon noted between the amounts of urea in the blood and the severity of the symptoms in patients, and later workers, Frerichs, in 1852, Feltz and Ritter, in 1880, Bouchard, in 1887, Herter, in 1898, and many others, found that urea was relatively nontoxic, when injected into animals. In fact, Bouchard, using rabbits, found that the amount of a concentrated urea solution necessary to produce severe symptoms was equal to the toxic dose of distilled water itself. Although Gallois, Richardson, in 1862, Voit, in 1868, and others, claimed positive results,

* From the Department of Pathology, University of Chicago, and the Otho S. A. Sprague Memorial Institute.

1. For most of the literature up to 1900 we have made use of Ascoli's "Urämie" (1902) in which a complete review of the subject is given.

they were met with the objection that their results were due, not to the urea itself, but to various impurities or to the osmotic changes induced by the concentrated solutions employed.

An anatomic theory, suggested by Traube in 1860, ascribed uremia to edema of the brain and consequent circulatory disturbances. But necropsies soon disclosed the fact that great inconsistencies existed between the anatomic and clinical findings, so the theory lost ground, and a return to a chemical theory followed. This time ammonium carbonate was blamed, Frerichs, in 1851, postulating that urea in the blood was converted by ferments into the toxic ammonium compound, while Treitz, in 1859, believed that the change occurred in the intestines. However, the ammonia content of uremic blood was found to be negligible.

Voit, in 1868, Feltz and Ritter, in 1881, and Linbeck, in 1898, turned their attention to the inorganic constituents of urine, and chose potassium chlorid, because of its extreme toxicity to animals, as the cause of the symptoms of uremia—especially the convulsions and coma. However, the clear-cut cardiac picture in potassium poisoning, and the inability to demonstrate a constant increase in the potassium content of the uremic blood, spoke against this theory. Schottin, in 1853, found an increased amount of extractives in the blood of uremics—especially creatin and creatinin. Jaccoud, in 1867, had a similar experience, and Landois, in 1891, had worked up the subject very thoroughly and produced convulsions in rats, dogs and apes by the direct application of creatin to the cerebral cortex. Feltz and Ritter, however, had to inject 2 gm. creatinin intravenously before they could produce convulsions and death, and these they obtained only in one dog. Various salts of creatin and creatinin, in from 5 to 6 gm. doses, had no effects. Various other extractives—uric acid, hippuric acid, leucin, tyrosin, purin bases, phosphates, sulphates and chlorids—were similarly ruled out as a possible cause of uremia.

Still clinging to the idea of urinary retention as the cause of uremia, Bouchard, from 1882 to 1889, worked on the toxicity of whole urine when injected into rabbits. He found human urine quite toxic, and could account for only half of this toxicity on the basis of potassium salts. He could extract various substances from human urine, which, on injection into rabbits, produced any or all of the symptoms of uremia. The urine of nephritic patients was found to be much less toxic than normal urine—due, presumably, to retention of the toxic products. Bouchard calculated that in twenty-four hours an average man excreted sufficient toxins in his urine to kill him in fifty-two hours, were all these toxins not eliminated. But Fowler, in 1881, reported many cases of complete mechanical obstruction to the outflow of urine, the patients living for many days and even two weeks without any other symptoms than asthenia and drowsiness; hence,

there was a great difference between uremia and anuria. Bouchard's work, on the whole, was very crude, and it was to be expected that he could obtain almost any symptoms by injecting such a complex of unknowns and unknowables as is present in human urine.

Brown-Sequard, in 1889, published his theory on internal secretions and included the kidney as one of the organs of internal secretion, ascribing uremia to failure or lack of such a secretion. In 1893, he stated that while nephrectomized animals lived only thirty hours on the average, similar animals to whom kidney extracts were given lived sixty hours. Meyer, in 1893 and 1894, reported that renal extracts had a marked effect on the pulse and respiration of anesthetized animals that were nephrectomized, changing the respiration from Cheyne-Stokes to normal. These results were obviously trivial and unimportant, and the theory of internal secretion of the kidney was dropped for a time.

Ascoli could see no very close relationship between the nonprotein nitrogen of the blood and the severity of symptoms, although some parallelism might be made out. Perhaps, both are merely the results of nephritis and, hence, a causal relationship between the two need not exist. In 1902, Ascoli described nephrolysins—substances having specific toxic effects on the kidneys, with side effects on nervous tissue, substances akin to immune bodies and produced by using renal tissue as antigen—and even isonephrolysins, produced when a kidney is injured for some reason or other,—and explained all the symptoms of what he called "true uremia without retention"—in contrast to "urinary intoxication with retention"—on the basis of a general intoxication with nephrolysins. He believed that nephrolysins could cause the increased blood pressure, the localized palsies, the edema, the pericarditis, stomatitis and colitis, the convulsions, the coma—in short, the classical picture of a clinical uremic attack. Ascoli emphasized especially the difference between the uremia resulting from mechanical obstruction of the urinary tract and the uremia found as a terminal event in cases of chronic nephritis—something which previous investigators had not quite clearly understood, and which was the cause of much unnecessary dispute and inconsistent experimental work. Every author had a different idea of uremia, and it is no wonder that no two authors agreed in their results.

With the advent of physicochemical investigations, many men turned their attention to the relationship between physicochemical changes in the blood and uremia. Honigmann [2] reviewed the literature in 1902. Koranyi found that the molecular concentration of the blood increased in uremia—using cryoscopy. Lindemann found a definite increase in the depression of the freezing point of water (Δ) of the blood of

2. Honigmann: Ergeb. d. Pathol. **8**:549, 1902.

uremic patients and dogs with both ureters ligated, and believed that the change in osmotic pressure was the chief cause of trouble. In an exhaustive monograph, Strauss, in 1902, found an increased Δ of the blood—usually in chronic interstitial nephritis—and a retention of organic molecules till the limit of the patient's tolerance to poisonous metabolites was reached, when any one or several of many factors might produce the uremic intoxication. Bickel, in 1902, found no change in the electrical conductivity of the blood of nephrectomized animals, and, with an increased Δ, he concluded that there must be a retention of organic molecules. However, in all these cases, no constant relationship could be found between the changes in Δ and other physical constants and the severity of the symptoms, so that, as Honigmann correctly concluded, no single series of facts—chemical, physical, or otherwise—had yet stood in constant parallelism with the clinical picture of uremia. The new findings were simply concomitant symptoms, so to speak, and not etiologic factors. Yet Stern [3] was willing to ascribe uremia to diminished electrical conductivity of the blood serum.

Bradford, in 1899, excised varying amounts of renal tissue in dogs, and found an increased output of nitrogen in the urine—due, he thought, to increased nitrogenous catabolism in the muscles. Others misinterpreted his views as favoring an internal secretion for the kidney. Pearce,[4] in 1908, removed one half, two thirds and three fourths of the renal tissue in dogs, but found no change in nitrogenous metabolism. But removal of more than three fourths of the total renal tissue led to metabolic upset, owing to starvation and gastro-intestinal disturbances. This work was well controlled, and the further work of Pearce [5] and Pearce and Sawyer [6] helped to disprove completely two theories—the internal secretion theory and Ascoli's nephrolysin hypothesis. Pilcher, in 1913,[7] extirpated renal tissue by ligation of the renal vessels—instead of excision of kidney tissue—and obtained results entirely confirmatory of Pearce's work.

When theories begin to fail, people turn their attention to facts. In scientific work much more progress may be made by the invention of new methods than by modifications of old ones, and so, in the work on uremia and chronic nephritis, the development of, perhaps, the most interesting phase of the problem—the retention or accumulation of waste products, especially nitrogenous, in the blood—was made possible by the new, delicate methods of blood and urine analysis

3. Stern: Med. Rec. **63**:121, 1903.
4. Pearce: J. Exper. M. **10**:632. 1908.
5. Pearce: Arch. Int. Med. **2**:77 (July) 1908.
6. Pearce and Sawyer: J. M. Res. **19**:269. 1908.
7. Pilcher: J. Biol. Chem. **14**:389, 1913.

introduced by Folin and his collaborators[8] since 1912, and extended by Marshall,[9] Van Slyke and Cullen,[10] Myers and Fine,[11] and many others. As a result, much of the older work has been discounted, and advantage is taken of the new methods by clinicians and laboratory men alike, in order to help accumulate sufficient data from which to draw definite conclusions.

It was suspected very early in the nineteenth century that a retention of nitrogenous waste products occurred in the blood in nephritis. The methods of investigation, however, were very crude, and the results inaccurate. Among those who worked on the nitrogen content of the blood of uremic patients, ordinary nephritics and experimental animals, were Wilson, Bostock, Frerichs, Treitz, Ascoli, Van Noorden, Schotten, Oppler, Hoppe-Seyler, Jaccoud, Landois, and a host of others.[12] Strauss, in his monograph, gave the nonprotein nitrogen of normal blood as from 20 to 35 mg. per 100 c.c.; in chronic parenchymatous nephritis, 40 mg. or over; in chronic interstitial nephritis, 85 mg. and over, reaching higher values, in uremia. Hohlweg, in 1911,[13] found 41 to 60 mg. of "residue" nitrogen per 100 c.c. in normal sera, of which 60 per cent. was urea nitrogen, as high as 120 mg. in nephritis, and up to 340 mg. in uremia. Other conditions showed no such changes. Foster, in 1912,[14] found from 43 to 164 mg. nonprotein nitrogen in chronic nephritis; from 53 to 393 mg. in uremia, with the urea nitrogen varying from 36 to 90 per cent. of the nonprotein nitrogen. Nonprotein nitrogen of 100 mg. (per 100 c.c.) or more was considered a poor prognostic omen.

With newer methods, the following results were obtained: Folin and Denis, in 1913,[15] found, in normal people, nonprotein nitrogen from 22 to 26 mg.; urea nitrogen, from 11 to 13 mg.; in chronic nephritis, nonprotein nitrogen of from 40 to 96 mg.; urea nitrogen of from 19 to 68 mg. Farr and Austin, in 1913,[16] found, in nonnephritic cases nonprotein nitrogen from 15 to 43 mg. with urea nitrogen of from 50 to 60 per cent. of the former; no change in uncomplicated cardiovascular cases, some increase in chronic parenchymatous

8. Folin and Farmer: J. Biol. Chem. **11**:493, 1912. Folin: Ibid. **11**:507, 1912. Folin and Macallum: Ibid. **11**:523, 1912. Folin and Denis: Ibid. **11**: 527, 1912.

9. Marshall: J. Biol. Chem. **14**:283, 1913; ibid. **15**:487, 1913; ibid. **15**: 495, 1913.

10. Van Slyke and Cullen: J. Biol. Chem. **19**:211, 1914; ibid. **24**:117, 1916.

11. Myers and Fine: J. Biol. Chem. **20**:391, 1915.

12. Obermayer and Popper: Ztschr. f. klin. Med. **72**:332, 1911.

13. Hohlweg: Deutsch. Arch. f. klin. Med. **104**:216, 1911.

14. Foster: Arch. Int. Med. **10**:414 (Sept.) 1912.

15. Folin and Denis: J. Biol. Chem. **14**:29, 1913.

16. Farr and Austin: J. Exper. M. **18**:228, 1913.

nephritis; nonprotein nitrogen from 40 to 180 mg. in chronic nephritis with hypertension. The nonprotein nitrogen was usually, but not constantly, high in uremia. Tileston and Comfort, in 1914,[17] studied 142 cases of nephritis and many other conditions—the latter as controls—and considered as the normal nonprotein nitrogen 30 mg. or less, with 50 per cent. urea nitrogen. In uremia, the nonprotein nitrogen was as high as 324 mg., urea nitrogen, 70 per cent. or more. Nonprotein nitrogen over 100 mg. was practically never found by them in conditions other than uremia; when occurring it was a very serious sign, and was apt to be followed very soon by death. Patients with a high nonprotein nitrogen were poor surgical risks.

McLean and Selling, in 1914,[18] gave the normal nonprotein nitrogen as from 23 to 36 mg. per 100 c.c., the urea nitrogen as from 10 to 23 mg. Foster, 1915,[19] found an average nonprotein nitrogen of 63 mg. in parenchymatous nephritis, with 84 mg. in the chronic interstitial variety, and 135 mg. in "convulsive uremia." The uric acid—normally from 3 to 5 mg. per 100 c.c.—averaged 12 mg. in uremic patients, but there was no concordance between the rise in purine and nonprotein nitrogen. In four convulsive cases, the creatin and creatinin ran from 27 to 46 mg. per 100 c.c., indicating a rather marked retention. Cullen and Ellis, 1915,[20] compared the urea nitrogen in blood serum and spinal fluid respectively, and found in the former a range of from 20 to 42 mg., in the latter from 22 to 46 mg., a very close correspondence, it is seen, and indicating the marked diffusibility of urea.

Schwartz and McGill, 1916,[21] studying the blood urea, gave, as the normal for human blood, 25 mg. urea per 100 c.c.; in acute nephritis, twenty-five cases, an average of 57.8 mg. was obtained; in mild cases of chronic nephritis, 28.8 mg. with 44.4 mg. in advanced cases, and 52.3 mg. in advanced cases with complicating cardiac decompensation. The urea content of blood and other body fluids was found to be very similar. Kast and Wardell,[22] 1918, in a study of 244 hospital cases with a urea nitrogen of less than 35 mg., concluded that 20 mg. was the upper normal limit for blood urea nitrogen, thus agreeing pretty closely with most of the previous figures. Finally, Gettler and St. George,[23] 1918, in a review of 600 cases of primary nephritis, found the nonprotein nitrogen to range between 40 and 460 mg. as compared with the normal of from 25 to 40 mg., while the

17. Tileston and Comfort: Arch. Int. Med. **14**:620 (Nov.) 1914.
18. McLean and Selling: J. Biol. Chem. **19**:31, 1914.
19. Foster: Arch. Int. Med. **15**:356 (Sept.) 1915.
20. Cullen and Ellis: J. Biol. Chem. **20**:511, 1915.
21. Schwartz and McGill: Arch. Int. Med. **17**:42 (Jan.) 1916.
22. Kast and Wardell: Arch. Int. Med. **22**:581 (Oct.) 1918.
23. Gettler and St. George: J. A. M. A. **71**:2033 (Dec. 21) 1918.

urea nitrogen, normally from 10 to 18 mg., ranged from 20 to 375 mg. In 120 cases of gout, the nonprotein nitrogen was from 38 to 55 mg. and urea nitrogen from 15 to 35 mg.—somewhat increased. They stated that where the nonprotein nitrogen was 80 mg. or above, increased protein catobolism, or failing, or poor, circulation could not be blamed as the cause, but the only possible cause was some renal trouble.

An interesting fact was pointed out by Chace and Myers,[24] 1916, who showed that in the various stages of a clinical nephritis, uric acid was retained first, then urea and, last of all, creatinin, so that one might have uric acid from 6.1 to 9.5 mg. with normal urea and creatinin, giving a "steplike" picture. In fatal cases, the uric acid ranged from 8.7 to 22.4 mg., urea nitrogen from 144 to 263 mg., and creatinin from 11.0 to 22.2 mg. All patients with a blood creatinin of more than 5 mg. died in a short time. They record thirty-four cases in which the creatinin ranged from 4.9 to 33.3 mg. Myers and Killian,[25] 1916, reported ninety-four cases with creatinin values of more than 5 mg., of which number eighty-three patients had died in a short time—80 per cent. in less than two months. They believed that the blood creatinin determination furnished a most reliable prognostic test in nephritis or uremia.

From the experimental side, it is interesting to note that in uranium nephritis—in cats—a marked retention of nonprotein nitrogen and urea nitrogen occurs; so also in cantharidin nephritis and less so in chromate nephritis, as reported by Folin, Karsner and Denis,[26] in 1912. Later on, in 1914, Karsner and Denis [27] produced a marked retention in acute nephritis as the result of injections of specific hemolytic immune serum, diphtheria toxin, and, especially, tartaric acid—nonprotein nitrogen 382 mg. in one cat. Tubular changes seemed a little more important in producing retention than glomerular changes, but definite conclusions could not be drawn. Removal of from one half to two thirds of the total kidney substance resulted in only a slight nitrogen retention for from twenty-four to seventy-two hours, but complete nephrectomy was followed by a progressive increase in nonprotein nitrogen of the blood—the values ranging from 227 to 285 mg. in three dogs reported by Karsner and others,[28] 1915.

Another evidence of retention in chronic nephritis and uremia is afforded by the study of the blood indican. Obermayer and Popper,[12] in 1911, believed that the presence of indican in the blood was specific

24. Chace and Myers: J. A. M. A. **67**:929 (Sept. 23) 1916.
25. Myers and Killian: Proc. Soc. Exper. Biol. & Med. **16**:41, 1918.
26. Folin, Karsner and Davis: J. Exper. M. **16**:789, 1912.
27. Karsner and Denis: J. Exper. M. **19**:259, 1914.
28. Karsner, Bunker and Grabfield: J. Exper. M. **22**:544, 1915.

for uremia. In thirty-eight uremic patients they found a blood indican of from 3.7 to 6.0 mg. per 100 c.c. They did not ascribe any etiologic rôle to indicanemia, but considered it a delicate indicator of retention. Tschertkoff,[29] in 1914, found indicanemia in many cases of nephritis without uremia. Haas,[29] in 1914, using better methods, determined 0.045 mg. per 100 c.c. as the amount of indican in the blood of normal individuals, from 0.06 to 0.145 mg. occurring in gastro-intestinal affections, from 0.2 to 2.7 mg. in nephritis with definite "renal insufficiency." Indican was increased long before the nonprotein nitrogen and was a better indicator of impending "renal insufficiency" than either the nonprotein nitrogen or urea nitrogen—a value of 0.180 mg. being a very suggestive sign of impending trouble. However, injection of large doses of indican into dogs and man—up to 40 mg.—proved perfectly harmless, as might be expected.

It was early discovered that in the late stages of renal diseases there occurs a state of acidosis, or decreased alkali reserve of the blood. Jaksch,[2] in 1900, found a decreased alkalinity of the blood in uremia. Orlowsky,[2] 1902, found a similar change in dogs with both ureters ligated. This was considered as simply another evidence of retention. Straub and Schlayer [30] believed acidosis to be an important factor in the production of uremia, for they obtained an alveolar carbon dioxid tension of less than 35 mm. Hg., in eight cases of uremia. No acetone bodies were found. Peabody,[31] in 1915, found an acidosis—determined by the bicarbonate tolerance method of Sellards— in advanced cases of chronic nephritis; in very advanced cases the alveolar carbon dioxid partial tension fell. This acidosis was secondary to retention, and very constant in uremia. In only a few cases, however, did it cause a definite air-hunger or other symptoms. Sellards [32] had previously obtained very similar findings. Somewhat at variance with other investigators, Fischer,[33] in 1912, went as far as to call acidosis the cause of nephritis. In 1916, Marriott and Howland,[34] following the finding by Greenwald of increased total phosphorus in the serum of nephritics, reported fourteen cases of chronic nephritis and thirty-five normal people on whom blood studies had been made regarding the nonprotein nitrogen, urea nitrogen, inorganic phosphorus, calcium and magnesium. In normal people, the total inorganic phosphorus was usually less than 2 mg. per 100 c.c.; in

29. Haas: Deutsch. Arch. f. klin. Med. **119**:117, 1916.

30. Straub and Schlayer: München. med. Wchnschr. **59**:569, 1912.

31. Peabody: Arch. Int. Med. **16**:955 (Dec.) 1915.

32. Sellards: Bull. Johns Hopkins Hosp. **23**:289, 1912; ibid. **25**:141, 1914; Principles of Acidosis, 1917.

33. Fischer: Edema and Nephritis, 1912.

34. Marriott and Howland: Arch. Int. Med. **18**:708 (Dec.) 1916.

chronic nephritis without acidosis, it was from 2.7 to 5.5 mg.; in chronic nephritis with acidosis, it ranged from 8 to 23 mg. They concluded that acidosis in nephritis was due to retention of acid phosphates —a condition not found in diabetic acidosis. The rather low calcium content found might be related to the convulsions and hemorrhages of uremia; the increased phosphorus probably caused an increased excretion of calcium phosphate into the intestine.

As regards other changes in the blood, than those described above, Gabbi [2] described an increased serum globulin in rabbits and dogs with both ureters ligated. Recently, in 1916, Butterfield and others [35] studied the blood of normal people and those with cardiac and renal decompensation and edema, uremia and hypertension, for the Δ, refractive index, specific gravity, dry residue and protein content. In chronic nephritis with uremia, Δ was from 0.61 to 0.76 degrees—quite high as compared with the normal Δ—0.57; the dry residue was increased, and especially the total protein content, i. e., the serum was concentrated. When edema was also present, all the findings but Δ were lowered, due to dilution. Cases of cardiac decompensation with edema showed normal values. Rowe,[36] in 1917 found, in chronic nephritis with edema, the lowest values for total serum proteins of those found in any disease; however, the globulin was increased. Bienenstock and Csaki,[37] in 1917, made an extensive series of observations on the physicochemical changes in the blood of dogs in whom both ureters were ligated or both kidneys removed. Δ was increased in all cases, up to 0.771 degrees; the electrical conductivity of the serum was decreased; the organic molecules were increased, the serum ash was increased; the fluidity of the blood varied, as also the viscosity, surface tension and total solids. The carbon dioxid in the serum was decreased, but markedly increased in the red blood corpuscles. The H-ion concentration of the serum rose—due to undetermined organic acids.

Two suggestive studies have been made on the presence of some very toxic substance in the blood or urine. Foster,[38] in 1915, isolated a toxic base from the blood of seven uremic patients—not found in twelve controls—which caused death in guinea-pigs, preceded by dyspnea, twitchings, convulsions, coma and subnormal temperature. At the necropsy, there were found hyperemia of the brain and kidneys. Hartman,[39] in 1915, isolated an organic compound, C_6H_8O, which he called "urinod," obtainable from urine, from 1 to 2 parts in 100,000.

35. Butterfield et al.: Am. J. M. Sc. **151**:63, 1916.
36. Rowe: Arch. Int. Med. **19**:354 (Feb.) 1917.
37. Bienenstock and Csaki: Biochem. Ztschr. **84**:210, 1917.
38. Foster: Tr. Assn. Am. Phys. **30**:305, 1915.
39. Hartman: Arch. Int. Med. **16**:98 (July) 1915.

Urinod was very toxic, producing symptoms very much like those of uremia, and having the typical "urinous" odor of a uremic patient's breath. The symptoms reproduced in man and animals were chiefly of the nervous type.

The clinical classification of uremia has varied from time to time as new facts were established and old ones confirmed or disproved. Thus, Widal,[40] in 1912, described two chief types of uremic syndromes—the "chloruremic" variety, associated with retention of sodium chlorid and with symptoms of edema, diarrhea, vomiting, coma, Cheyne-Stokes breathing, delirium, convulsions and amblyopia; and the "azotemic" type in which nonprotein nitrogen retention was marked, with gastrointestinal disturbances—anorexia, vomiting, ulcerative stomatitis and colitis, pericarditis, albuminuric retinitis, stupor and torpor—with mixed pictures resulting from the predominance of urinary, vascular or cardiac syndromes.

Reiss,[41] in 1914, in an excellent review of the subject, attempted to classify uremia on a symptomatic basis, and gave three main types—the "asthenic uremia," in which predominating symptoms were drowsiness, fatigue, apathy, indifference, physical weakness, marked nonprotein nitrogen retention, and death, usually from rather sudden cardiac failure; the "convulsive or epileptiform" type, in which convulsions, unconsciousness, a high blood pressure, with little or no disturbances in the urinary secretion and practically no nitrogenous retention were characteristic symptoms; the "psychotic uremia," in which psychic symptoms, such as dementia, hallucinations, delusional insanity, deep coma and muscle twitchings, occurred, with nonprotein nitrogen not necessarily increased, but with evidence of cerebral arteriosclerosis in the majority of cases; and, finally, a mixed group in which asthenic and convulsive features were combined—by far the largest clinical group. In the asthenic variety alone were nonprotein nitrogen and sodium chlorid retention marked and characteristic. Reiss believed that the nonprotein nitrogen retention was the cause of asthenic uremia. In the other types, it could be stated definitely that the toxic substances did not arise from disturbances in the uirnary tract, but were metabolic in origin.

Strauss,[42] in 1915, reserved the term "true uremia" for cases with definite retention—nonprotein nitrogen, 150 mg. or more. All the other forms, including the convulsive and arteriosclerotic types, he grouped under "pseudo-uremia"—with nonprotein nitrogen below 70 mg. Most authors now agree in recognizing at least two types of uremia—the

40. Widal: Presse méd. **20**:973, 1912.
41. Reiss: Ztschr. f. klin. Med. **80**:97, 1914.
42. Strauss: Berl. klin. Wchnschr. **52**:368, 1915.

asthenic and the toxic. Heim and Tchertkoff,[43] in 1918, described three types—one due to chlorid retention, a "true uremia" due to urea retention, and "pseudo-uremia"—the latter associated with general arteriosclerosis, and no retention, usually found after 40 years of age.

The finding of increased nonprotein nitrogen in the blood of uremic patients, and the fact that from 30 to 90 per cent. of this nitrogen was urea nitrogen, naturally led to the thought that urea was toxic. Experimentally, it was shown that double nephrectomy or ligation of the ureters produced a rise in blood urea. Thus, Herter and Wakeman,[44] in 1899, removed both kidneys in the dog, with resulting subnormal temperature, anorexia, drowsiness, vomiting and diarrhea, and death in from twenty-two to eighty-two hours. Ligation of both ureters gave similar results. In twenty-eight normal dogs, the blood urea was 37 mg. (average); in eighteen nephrectomized animals, it was as high as 315 mg. (average), and 307 mg. in animals with both ureters ligated. There were also an increase in the total proteins of the blood, and in the total phosphorus. The tissues, in general, contained from four to five times the normal percentage of nitrogen—probably urea. It might be said that earlier work on the subject is open to criticism because of the great inaccuracy of the analytical methods in vogue.

In 1914, some definite work on the toxicity of urea was done by Marshall and Davis,[45] using accurate but simple methods. Vauquelin and Segales, in 1822, were the first to record intravenous injections of urea in animals. They used small amounts and obtained negative results. Hammond, in 1858, interfered with the kidneys while injecting, and found the injections fatal. Grehant and Quinquard, in 1884, determined 600 mg. per 100 c.c. blood as the lethal dose for dogs. Herter and Wakeman, in 1899, caused the death of normal dogs by using concentrated urea solutions; urea to the extent of 1 per cent. of the body weight had to be injected to produce fatal effects. Marshall and Davis studied the distribution of injected urea, in the blood, urine, bile, spinal fluid and tissues. In normal dogs, the blood urea was from 21 to 29 mg. per 100 c.c., while the urea content of other body fluids and tissues was very similar to these figures, except in the case of fat, which was poor in urea, and the urinary tract, which was obviously rich in urea.

By injecting increasing doses of urea, in concentrated solutions, into dogs on successive days they produced, in the order named, loss of appetite, vomiting and restlessness, convulsions, coma and dyspnea, and death—the latter occurring when the amount injected reached 1 per cent. of the body weight, e. g., 25 gm. in a 2.7 kg. dog. The urea

43. Heim and Tchertkoff: Rev. méd. Suisse Rom. **38**:15, 1918.
44. Herter and Wakeman: J. Exper. M. **4**:117, 1899.
45. Marshall and Davis: J. Biol. Chem. **18**:53, 1914.

injected was excreted rapidly, and what remained was fairly evenly distributed in the blood and tissues of the body—as one would expect from the ready diffusibility of urea. There was no evidence that any of the urea was changed into other compounds.

In some animals one ureter was ligated, and it was found that, after a short time, the remaining functioning kidney could take care of as much urea as both kidneys previous to the operation, indicating a high margin of safety.

It was shown, both by injecting single large doses and repeated small doses of urea, that the tissues took up about 90 per cent. of the injected urea in a few minutes. Tremors and opisthotonic convulsions were produced when large doses were injected. At the necropsy, the brain was found to be wet and hyperemic.

Marshall and Davis concluded that the concentration of urea in the blood did not affect the ability of the tissues to absorb urea; that the kidneys had an enormous power of excreting urea—up to 40 gm. per liter; that dehydration retarded elimination, and that, in man, urea accumulated only slowly in the blood, owing to absorption by the tissues, so that it would take from four to six days of complete retention to raise the blood urea to 200 mg. Analysis of human tissues, from cases of nephritis, showed a high urea content, closely analogous to that found in the dogs. Urea was toxic only in large doses—1 per cent. of body weight—but produced symptoms in smaller amounts. It was not transformed into other compounds.

As regards the toxicity of urea in man, when urea is ingested, Hewlett and others,[46] in 1916, carried out some interesting experiments. One hundred gm. of urea, in aqueous solution, were swallowed in fifteen minutes, or in divided doses, over a period of from four to six hours. Symptoms of headache, dizziness, apathy, drowsiness, fatigue and inability to work appeared, and were most marked when the blood urea was at its maximum—from 150 to 160 mg. per 100 c.c. There were practically no symptoms between 40 and 150 mg., and symptoms always appeared after 150 mg., persisting till the blood urea had again fallen below that level. Hence, the sudden entrance of urea into the blood was not the cause. The authors noted the analogy between the symptoms produced and those seen in asthenic uremia, or in the later stages of nephritis when retention occurred.

Following a thorough review of the literature on uremia, Wells,[47] in 1920, was inclined to the view that uremia was caused by the retention of the known nitrogenous substances in the blood, emphasizing especially the importance of the time element, for the presence in the blood of a high concentration of organic substances, continuously main-

46. Hewlett, Gilbert and Wickett: Arch. Int. Med. **18**:637 (Nov.) 1916.
47. Wells: Chemical Pathology, 1920.

tained, was bound to have deleterious effects, especially when one considered the marked symptoms following even a very temporary increase in nonprotein nitrogen, if 200 mg. or more. All the pathologic findings pointed to a systemic intoxication, and varying clinical pictures might very readily be produced by variations in the retention of the several known nitrogenous constituents. Experiments with the Woodyatt machine to determine the effects of prolonged intravenous injections of the various purified urinary constituents were recommended as being highly essential for further progress.

THE METHODS OF EXPERIMENTATION

Dogs were used. They were not restricted as to food or drink. Injections of urea were made intravenously, using a buret, rubber tube and hypodermic needle, usually without cutting down on the vein—the external saphenous or some superficial thigh vein—unless absolutely necessary, and then under local anesthesia. A 33⅓ per cent. solution of urea was always used, at body temperature. No general anesthetic was necessary since the dogs lay quietly on the table, except when convulsions occurred. Blood was drawn from a leg vein, by means of a hypodermic needle attached by short rubber tubing directly to an Ostwald pipet, so that suction could be applied. In the first few dogs, the urine was obtained by means of a catheter, but later on, owing to the difficulty of inserting a catheter without assistance, and because it was not possible always to obtain female dogs, the catheter was abandoned, and samples of urine obtained only during urination and at necropsy. No attempt was made to collect twenty-four hour samples of urine.

The injections were usually continuous, except for the time it took to fill a 50 c.c. buret, and were continued until the twitchings or convulsive movements of the animal made injection difficult. Symptoms were noted carefully, at varying intervals. Necropsy was performed on all the dogs within a few hours after death, and samples of heart's blood, urine, bile, gastric or intestinal contents, taken for analysis. The viscera and the brain and spinal cord were examined in a routine manner and hematoxylin and eosin celloidin sections, as well as frozen sections for fat stains, were made.

In cases in which operative procedures were necessary—in ligating the ureters—strict aseptic precautions were observed and the anesthetic, ether, was administered by an assistant. A midline abdominal incision was employed, except in one case.

The urea in the blood was determined by mixing the drawn blood with 1 c.c. 5 per cent. sodium citrate and diluting, or not, with distilled water, if necessary, so as to give from 1 to 3 mg. of urea in 5 c.c. of the mixture or whole blood. In a large test tube, 5 c.c. of whole or diluted blood were placed, 5 c.c. of water added, 1 c.c. of 10 per cent. urease solution (freshly prepared from "Arlco" urease) and a few c.c. of paraffin oil (later a few drops of caprylic alcohol were substituted), and allowed to stand at room temperature for at least 30 minutes. This tube was connected with another tube containing from 15 to 25 c.c. of hundredth normal hydrochloric acid, some 1 per cent. solution of sodium alizarin sulphonate, and paraffin oil as above—the set of two tubes constituting part of a complete aeration apparatus, with a water suction pump to create a negative pressure. Usually, several sets of tubes were run in series. After standing thirty minutes, suction for a minute or two was carried out to get any free ammonia vapor into the acid tube; then 10 c.c. of a 50 per cent. suspension of anhydrous, finely-powdered sodium carbonate were introduced into the tube containing the fluid to be analyzed, and suction carried out for at least thirty minutes, at first a slow current for from two to three minutes and then a gradually increasing current until the maximum

aeration was obtained. Titration of the acid tube with hundredth normal sodium hydroxid and sodium alizarin sulphonate, as indicator, was done, and the urea readily estimated.

For urine, the ammonia content was first determined by simply adding 10 c.c. of a 15 per cent. potassium carbonate and 15 per cent. potassium oxalate mixture to 1 c.c. of urine, and aerating. The ammonia nitrogen was subtracted from that found in the estimation of urea. Urine was usually diluted 1:100, and so also bile and other body fluids, before determining the urea content.

Experiments with known solutions of urea were carried out until accurate results were obtained with the urease and titration method. Controls were, of course, run on the blood and other fluids for ammonia nitrogen not derived from urea, and all the reagents were carefully controlled, so that the figures given below represent the completely corrected figures. Urea was usually injected at the rate of 2 gm. per minute.

RESULTS

Owing to lack of space, the protocols of only a few of the experiments in each series are given in detail, while the remainder are abbreviated so as to include only the essential features of each experiment and any points of difference in the results of the various experiments carried out.

April 11, 1920.—Dog 1, brown female, 9.5 kg., received 30 gm. urea intravenously; vomited a few times. Blood urea rose from 20.7 mg. per 100 c.c. to 348.3 mg. No after-effects were noticed.

April 11, 1920.—Dog 2, white female, 16.3 kg., showed no symptoms during or after the injection of 20 gm. urea, while the blood urea rose from 24.6 mg. to 161.4 mg. per 100 c.c.

April 15, 1920.—Dog 2 received 80 gm. urea. Nausea and salivation occurred after 40 gm. urea were injected; dyspnea and vomiting after 70 gm. Blood urea rose from 22.7 mg. to 491.4 mg. Urine urea was 1,780 mg. per 100 c.c. at the end of the experiment. The animal seemed well the next morning.

April 26, 1920.—Dog 2, white female, weighing 16.6 kg.; previously injected.

4:30 p. m.—Normal blood urea, 18.4 mg.; temperature 102.2 F.

4:50-5:17 p. m.—Injected 65.3 gm. urea (Merck). At 20 gm. dog was panting markedly (240 per minute); at 40 gm. salivation, nausea and vomiting, with dyspnea. Temperature 102.0 F.

5:30 p. m.—Blood urea, 536.4 mg.

5:47-6:15 p. m.—Injected 60 gm. urea.

5:50 p. m.—Dog quiet; respiration, 14 (per minute); heart, 66.

6:00 p. m.—Dog grunting; respiration, 48; heart, 102; temperature, 101.4 F.

6:30 p. m.—Urine bloody, no casts; 5,090 mg. urea per 100 c.c. urine.

6:55-7:15 p. m.—Injected 77 gm. urea. Total injected 202.3 gm.

7:01 p. m.—Dog dyspneic (144 resp.); very restless and irritable.

7:15 p. m.—Blood urea, 1,812 mg.

7:25 p. m.—Generalized tremors.

7:30 p. m.—Temperature, 97.2 F.; tonic, then clonic convulsions, affecting the whole body, lasting thirty minutes; then spasmodic jerking of the head and limbs, with paddling movements of the forelimbs for at least three hours. Frequent urination and bloody diarrhea; the animal was unconscious, the reflexes exaggerated, the breathing jerky and rapid.

9:00 p. m.—Temperature below 94 F.

April 27, 1920.—8:00 a. m. The dog still has jerky, opisthotonic movements of the head and neck, is in deep coma with slow respiration and slow pulse, no corneal reflexes, dilated pupils.

10:00 a. m.—Similar condition; deep coma; slow twitchings. Died between 11 and 12 a. m.

1:00 p. m.—*Necropsy.*—The whole small intestine was deeply injected; the mucosa of the stomach and small intestine was swollen, hemorrhagic and necrotic; the colon hyperemic; the kidneys large, tense, bluish with the cut surface pale and swollen, with fine grayish streaks in the lower half of the cortex, the medulla glistening; the heart with the right ventricle dilated, with subendocardial petechiae; the lungs collapsed, not crepitating, slightly boggy, multiple subpleural hemorrhages; the liver dark and full of blood; the gall-bladder distended with thick tarry bile, the brain and cord hyperemic and wet; the urinary bladder empty.

Histologic examination showed marked fatty degeneration (Sudan III frozen sections, hematoxylin as counter stain) in the limbs of Henle's loops and in the collecting tubules, with cloudy swelling of the convoluted tubules; central congestion and fatty degeneration in the liver; hyperemia of the intestinal mucosa, with mucoid degeneration of the epithelium and marked edema of the submucosa; areas of edema in the lungs, with subpleural hemorrhages.

Heart's blood urea at necropsy, 1,680 mg.

Bile urea at necropsy, 1,782 mg.

April 19, 1920.—Dog 3, brown female, 9 kg., received 115 gm. urea (under ether anesthesia) and developed generalized tremors, respiratory paralysis and death within fifteen minutes after the injection. Blood urea rose from 21.8 mg. to 1,125.6 mg. The necropsy findings were similar to those in Dog 2, but with hemorrhages in the stomach alone, and no visible changes in the liver. Bile urea at necropsy was 1,560 mg. per 100 c.c.; urine urea, 2,670 mg.

June 14, 1920.—Dog 10, brown female, 8.9 kg., received 120 gm. urea. Marked salivation, restlessness, tremors, tonic and clonic convulsions, opisthotonos, nystagmus, dyspnea, diarrhea, subnormal temperature, bloody urination and death resulted. Blood urea rose from 24.2 mg. to 1,644 mg. Urine urea rose to 3,230 mg. Necropsy showed the usual findings, very similar to those in Dogs 2 and 8. Heart's blood urea, 1,702 mg.; bile urea, 1,728 mg.

April 26, 1920.—Dog 6, brown and black female, weighing 13.9 kg.

4:10 p. m.—Temperature, 103.4 F.

5:15 p. m.—Normal blood urea, 21.5 mg.

5:40-6:30 p. m.—Injected 105 gm. urea in toto.

5:48 p. m.—After 15 gm., dyspnea (180 per minute); heart, 96.

5:54 p. m.—After 30 gm., vomiting of yellow mucus; respiration, 30; heart, 96.

6:03 p. m.—After 45 gm., visible peristalsis; temperature, 101.6 F.

6:05 p. m.—After 50 gm., salivation of a thick, viscid, stringy, mucous saliva, with 412.8 mg. urea.

6:15 p. m.—After 70 gm., deep labored breathing (48 per minute); heart, 180; rapid horizontal nystagmus, salivation, marked prostration.

6:18 p. m.—After 80 gm., vertical nystagmus, dyspnea, tremors of the limbs.

6:23 p. m.—After 85 gm., slight tremors of the head and neck; tendency to opisthotonos.

6:25 p. m.—After 90 gm., respiration, 144; dog almost chokes with thick mucus; stringy saliva pours from mouth.

6:30 p. m.—After 105 gm., backward jerkings of the head.

6:32 p. m.—Generalized clonic convulsions for one minute; animal in coma; corneal reflexes absent.

6:34 p. m.—Clonic convulsions for a few seconds; then sudden cessation of heart beat and respiration, with death. Temperature, 104.8 F.

6:50 p. m.—Blood urea at necropsy 669.5 mg.; urine urea, 3,690 mg. per 100 c.c.; bile urea, 1,260 mg.; gastric contents, 1,286 mg. urea per 100 c.c.; intestinal contents, 734 mg. urea (per 100 c.c.).

Necropsy showed some edema in the lungs; hemorrhages (submucous) in the stomach; hyperemia of brain and cord; the kidneys large, tense, bluish and dry on section, with grayish, cortical streaks.

Histologic examination showed marked fatty degeneration in the cortical rays, with cloudy swelling of the convoluted tubules of the kidneys; slight fatty changes in the liver, and a patchy congestion and edema of the lungs.

May 6, 1920.—Dog 7, black female, weighing 16 kg.

4:15 p. m.—Temperature, 104.8 F.; urine, 2,470 mg. urea per 100 c.c.; blood urea, 23.6 mg.

4:50-5:52 p. m.—Injected 94 gm. urea. At 45 gm., nausea, salivation, dyspnea; at 45 gm., vomiting; at 68 gm., much vomiting; respiration, 20; heart, 72; temperature, 101.2 F.

6:03-6:45 p. m.—Seventy-nine gm. urea injected. Total injected, 173 gm.

6:10 p. m.—Temperature, 101 F.; respiration, 24; heart, 66. At 136 gm., dog's mouth full of viscid, foamy saliva; jerky grunting respiration; at 170 gm., vomiting and several times thereafter.

7:25 p. m.—Blood urea, 1,452 mg.

7:30 p. m.—Temperature, 99 F.

8:30 p. m.—Temperature, 95.4 F.; dog restless, dyspneic, cannot stand, in stuporous state, conscious only of strong stimuli; died between 9 p. m. and 8 a. m.; found very rigid.

May 7, 1920, 11 a. m.—Necropsy showed hyperemia and hemorrhages (submucous) in the small intestine, pylorus, and the upper part of the colon; the left kidney large, flabby, cyanotic, with light gray streaks in the cortex; the right kidney half the size of the left, with knobby surface, depressed scars and adherent capsule, firm on cutting, with a large white scar in the pyramids and fibrous patches in the cortex; the heart with subendocardial hemorrhages in the right auricle; the lungs edematous, especially in the lower lobes and posteriorly, with bloody frothy fluid in all the bronchi; the brain and cord hyperemic and wet.

Histologic examination showed marked fatty degeneration in the cortical rays with cloudy swelling of the convoluted tubules of the kidney, scars of chronic interstitial nephritis in one kidney with hyaline casts; hypostatic congestion and edema in the lungs.

Blood urea at necropsy, 1,539 mg.; urine urea, 3,870 mg. per 100 c.c.; bile urea, 1,242 mg.; intestinal contents, 1,836 mg. urea.

May 17, 1920.—Dog 8, white female, weighing 11.5 kg.

4:03 p. m.—Normal blood urea, 11.9 mg.

4:03-5:08 p. m.—Injected 130 gm. purified urea.

[In order to eliminate ammonium salts as a possible cause of the symptoms, a quantity of urea (Merck's) was purified by recrystallization from alcohol, so that the solution of urea was practically neutral and less than 1 mg. ammonia nitrogen per 10 gm. urea could be obtained on aeration of a 33⅓ per cent. urea solution. Ordinary urea contained, on the average, 10 mg. free ammonia nitrogen per 10 gm. urea.]

4:20 p. m.—Temperature, 102. 6 F.; heart rate, 96; respiration, 14. At 30 gm. urea, salivation; at 60 gm. urea, nystagmus; dog seems depressed.

4:40 p. m.—At 75 gm. urea, very thick stringy saliva, containing 474 mg. urea; the animal's head was thrown back in opisthotonos.

4:50 p. m.—Urea, 90 gm.; slow backward jerkings of the head.

4:53 p. m.—Urea, 97 gm.; slight tremors of the head, marked opisthotonos and nystagmus.

4:56 p. m.—Urea, 102 gm.; slight generalized tremors, snapping movements of the jaws; temperature, 102.2 F.

5:02 p. m.—Urea, 116 gm.; marked generalized tremors; increased reflex irritability.

5:04 p. m.—Urea, 120 gm.; heart rate, 84; respiration, 20; shallow. Coarse twitchings and marked opisthotonos.

5:08 p. m.—Urea, 130 gm.; dog released; tries to stand, but has clonic convulsions and falls to the floor.

5:10 p. m.—Convulsions again; the animal rolls his head and body over and over, pants rapidly for one and one-half minutes, then tremors appear.

5:12 p. m.—Convulsions again for one-half minute; heart rate, 122; the animal goes into convulsions on being touched; convulsions and opisthotonos for three minutes following the insertion of a thermometer into the rectum; then sudden cessation of convulsions, with marked panting respirations and flaccid extension of the limbs.

5:20 p. m.—Temperature, 105.2 F.; respiration, 204; heart, 66 and weak.

5:33 p. m.—Blood urea, 1,242 mg.; dog breathing jerkily; in constant tremor and opisthotonos.

5:40 p. m.—Temperature, 102 F.

5:50 p. m.—Respiration, 78; heart, 66; temperature, 100.4 F.; generalized tremors; corneal reflex now present.

6:20 p. m.—Urinated 150 c.c. bloody urine; 3,730 mg. urea per 100 c.c.

6:55 p. m.—Temperature, 98.4 F.; respiration, 28; heart, 66; tremors.

7:45 p. m.—Temperature, 98 F.; no tremors; animal in stupor.

The animal died between 8 p. m. and 8 a. m., May 18, 1920.

May 18, 1920, 1 p. m.—Necropsy showed the small intestine hyperemic, with marked hyperemia and submucous hemorrhages in the stomach, duodenum and upper jejunum; the urinary bladder empty; the kidneys bluish, with injected cortical vessels and grayish streaks in the boundary zone; the liver pale and grayish on section; the heart with subendocardial hemorrhages in the left ventricle; the lungs with marked congestion and edema, and extensive petechial and purpuric extravasations under the pleura; the brain and spinal cord hyperemic.

Histologic examination showed very marked fatty degeneration in the cortical rays, with cloudy swelling of the convoluted tubules of the kidney; some peripheral fatty changes in the liver; hypostatic congestion and edema of the lungs.

Heart's blood urea at necropsy, 1,155 mg.; bile urea, 978 mg.

May 20, 1920.—Dog 9, brown female, 10.7 kg., with large goiter, received 118 gm. repurified urea and developed nystagmus, generalized twitchings, clonic convulsions, opisthotonos, dyspnea, bloody diarrhea, stupor, subnormal temperature, death; very similar to the picture described in Dog 8. Blood urea rose from 20.2 to 1,776 mg. Urine urea was 3,010 mg. at the end of the injection. Necropsy findings were similar to those in Dog 8, but with less marked lung involvement, fatty changes in the central parts of the liver lobules, and marked nodular and cystic goiter.

Heart's blood urea, 1,362 mg.; gastric contents' urea, 1,923 mg.; bile urea, 1,422 mg.

May 16, 1920.—Dog 5, black female, weighing 12.5 kg.; normal blood urea, 18.5 mg. The right ureter was doubly ligated, under ether anesthesia. Blood urea after operation, 22.5 mg.

May 24.—Blood urea, 21.3 mg.; the wound is superficially infected, the stitches loose; some were removed. The dog weighs 11.15 kg.

May 30.—Dog weighs 11.9 kg.; wound granulating well. Blood urea, 17.2 mg.

4:30 p. m.—Respiration, 24; heart, 126; temperature, 103 F.

4:40-5:25 p. m.—Urea, 87 gm. (Daigger), injected.

4:55 p. m.—At 30 gm., respiration, 24; heart, 104; temperature, 102.8 F.

5:00 p. m.—At 40 gm., signs of nausea.

5:07 p. m.—At 55 gm., ineffectual attempts at vomiting.

5:15 p. m.—At 70 gm., respiration, 28; heart, 100; temperature, 102.8 F.

5:18 p. m.—At 75 gm., tremors of the head and forelimbs; backward throwing of the head.

5:25 p. m.—At 87 gm., sudden vomiting, with tonic and clonic convulsions, opisthotonos, nystagmus; urinated 75 c.c. pale cloudy urine, containing 1.690 mg. urea per 100 c.c. The animal tries to get up, and keeps rolling his head over and over; paddling movements of the limbs.

5:33 p. m.—Convulsions cease for a few seconds, but begin again, lasting two minutes.

5:46 p. m.—Respiration stopped suddenly.

5:48 p. m.—Heart stopped beating.

6:15 p. m.—Necropsy showed the laparotomy wound to be clean on the peritoneal surface with a few adhesions; the distal end of the right (ligated) ureter was adherent to the small intestine; the proximal end dilated (1 cm.) and the pelvis much distended with pale, yellowish fluid so that the right kidney is twice the normal size, showing hemorrhagic necrotic areas in the medulla. Many white streaks in the cortex extending to the capsule as small solid pinhead sized projections; capsule not adherent; the left kidney somewhat enlarged, cyanotic, with light lower zone in the cortex; the lungs entirely collapsed; a few hemorrhagic areas in the pylorus and duodenum; the liver dark and full of blood; the brain and cord hyperemic.

Heart's blood urea, 978 mg.; bile urea, 1,185 mg.

Histologic examination showed fatty degeneration in the limbs of Henle's loops, cloudy swelling in the convoluted tubules of the left kidney, with the tubules dilated and containing protein material; hemorrhages and necrotic areas, and evidences of an ascending suppurative pyelonephritis in the right kidney; some fatty changes in the liver.

May 27, 1920.—Dog 4, white female, 12.8 kg., had the right ureter ligated. Blood urea rose from 20.8 to 23.8 mg.;

June 3.—Sixty-three gm. urea were injected, causing nausea, generalized tremors, tonic and clonic convulsions, nystagmus, dyspnea, diarrhea and stupor, from which the animal recovered, markedly depressed. Blood urea rose from 27.8 to 1,110 mg. Urine urea was 1,900 mg. at the end of the injection.

June 4.—The dog is depressed. Blood urea, 265.5 mg.

June 10.—Seventy-four gm. urea were injected, causing a picture similar to that noted June 3, with, again, recovery of the animal. However, the convulsions were much milder than previously. Blood urea rose from 20.1 mg. to 933 mg. Urine urea was 2,040 mg.

June 11.—The dog seems well. Blood urea, 106.8 mg.

June 14.—Blood urea, 13 mg.

June 26.—Blood urea, 24.7 mg. The left ureter was ligated, the bowel being perforated accidentally. Blood urea rose to 272.4 mg. The dog was much depressed and vomited frequently, dying on the third day. Necropsy showed a suppurative peritonitis, pyohydronephrosis on the right side, some hydronephrosis on the left side with necrosis of the kidney tissue, hypostatic congestion and edema of the lungs. Heart's blood urea, 356.7 mg.; bile urea, 369.3 mg.; gastric contents' urea 448.2 mg.

July 26, 1920.—Dog 14, brown female weighing 13.2 kg.; blood urea, 18.8 mg. The left ureter was ligated. Blood urea after operation, 30.6 mg.

July 31, 2:10 p. m.—Blood urea, 19.9 mg.; some wound infection; respiration, 36; heart, 150; temperature, 106.4 F.

2:15-2:53 p. m.—Injected urea, 75 gm.

2:30 p. m.—At 30 gm., respiration, 36; heart, 168; temperature, 105.8 F.

2:43 p. m.—At 56 gm., some nystagmus.

2:53 p. m.—At 75 gm., respiration, 36; heart, 36; temperature, 104.8 F. Some retraction of the head.

2:55-3:10 p. m.—Injected 30 gm. urea. Total injected, 105 gm.

3:02 p. m.—Increased irritability; nystagmus, contracted pupils, slight tremors of the head and neck, with twitchings of the forelimbs.

3:07 p. m.—Coarse twitchings of the head and neck, with opisthotonos.

3:08 p. m.—Tonic convulsions of the head, neck and forelimbs.

3:10 p. m.—More marked tonic convulsions, flexion of the head, bulging dilated eyes; the animal tries to stand.

3:15 p. m.—Generalized tonic convulsions.

3:14 p. m.—Opisthotonos, paddling movements, clonic convulsions, alternate flexion and retraction of the head.

3:17 p. m.—Convulsions continue; the animal defecates.

3:18 p. m.—Marked clonic convulsions, with opisthotonos, rolling movements, dyspnea with puffing out of the cheeks, diarrhea—lasting for two minutes.

3:26 p. m.—Paddling movements; opisthotonos.

3:30 p. m.—The animal is much quieter, but still has coarse tremors of the head and neck, with occasional paddling movements.

3:40 p. m.—Blood urea, 1.096 mg.

3:50 p. m.—The animal is panting; has much thick, mucus saliva; marked tenesmus and diarrhea.

8:00 p. m.—Animal is very much depressed and in a drowsy state.

August 1, 9:00 a. m.—Dog found dead.

Necropsy showed some omental adhesions around the wound; subserous hemorrhages in the small intestine, with submucous hemorrhages in the stomach and duodenum, and marked hyperemia and edema of the rugae of the colon; the left kidney one and one-half times larger than the right, very soft, with numerous clusters of yellowish pinpoint areas on the surface of the kidney, alternating with red raised areas, marked hemorrhages in the pyramids and cortex, with yellow streaks in the cortex and necrotic grayish areas in the medulla; the right kidney slightly enlarged, having a boiled appearance, with grayish streaks in the lower half of the cortex; the liver pale, with darker centers; the lungs collapsed and airless, with little fluid, not much subpleural hemorrhage; the brain and cord unchanged.

Histologic examination showed, in one kidney, the usual cloudy swelling and fatty degeneration; in the other kidney, an ascending suppurative process with hemorrhagic infarcts, necrosis and hemorrhage in the medulla and masses of organisms; some fragmentation of the myocardium; necrosis of the mucosa of the colon, with marked submucous edema; subpleural hemorrhages in the lung. Heart's blood urea, 1,224 mg.; bile urea, 1,263 mg.; gastric contents' urea, 1,863 mg.

July 29, 1920.—Dog 15, black and white female, 6.7 kg., had the right ureter ligated. Blood urea rose from 23.2 mg. before, to 29.1 mg. after, operation.

August 5.—Blood urea, 30.6 mg.; 75 gm. urea were injected, producing tremors, tonic and clonic convulsions, dyspnea, nystagmus, depression and death within sixteen hours. Blood urea rose to 1,275 mg.; urine urea to 1,730 mg. Necropsy showed the usual fatty changes in the kidneys, with necrosis of the convoluted tubules in one kidney, hemorrhages in the intestine, and some pulmonary congestion and edema. Bile urea, 1,659 mg.; gastric contents' urea, 1,768 mg.

June 23, 1920.—Dog 11, white and brown female, 6.2 kg., had both ureters ligated and cut. The dog lived less than thirty-six hours, showing dyspnea and depression. Blood urea rose from 22.6 mg. to 123 mg. Necropsy showed yellowish streaks and hemorrhagic areas in both kidneys, hemorrhages and a patch of bronchopneumonia in the lungs; microscopically, fatty changes in the liver and kidneys, with necrosis of the convoluted tubules. Heart's blood urea, 243 mg.; urine (in pelvis) urea, 609 mg.; bile urea, 273 mg.; gastric contents' urea, 350 mg.

June 30, 1920.—Dog 12, white female, weighing 6.1 kg.

7:30 p. m.—Blood urea, 29.5 mg. Both ureters were ligated and cut; the dog vomited after the operation.

9:30 p. m.—Blood urea, 32.6 mg.

July 1, 2:30 p. m.—The animal seems to be in good condition. Temperature, 102.4 F. Blood urea, 92 mg.

July 2, 9:00 a. m.—The dog is in fair condition, but eats very little. Temperature 101.6 F. Blood urea, 198.7 mg.

July 3, 10:00 a. m.—The animal is depressed and drowsy. Temperature, 100 F. Blood urea, 234.7 mg.

8:00 p. m.—The animal is very much depressed and drowsy. Temperature, 99.6 F. Blood urea, 366.9 mg.

10:30 p. m.—The animal is lying down in the cage, apparently in very poor spirits.

July 4, 9 a. m.—The dog is very depressed and unresponsive. Temperature, 100 F. Blood urea, 432.3 mg.

July 5, 9:00 a. m.—The dog was found dead.

Necropsy showed some stitch abscesses in the laparotomy wound, but with the peritoneal surfaces perfectly clean; both kidneys slightly enlarged, the capsules strip readily, the medulla pale, with the cortex showing grayish-yellow streaks in its lower half and, in one kidney, a few small hemorrhages; both kidneys of a brown color; the liver dark and bloody on section, with the centers of the lobules very dark in color; the stomach and the upper part of the small intestine with circumscribed areas of necrosis; the lungs collapsed anteriorly, with boggy purplish areas posteriorly and subpleural hemorrhages in some of the lobes; the brain and cord unchanged.

Histologically, the kidneys showed granular degeneration and necrosis of the convoluted tubules, with abscesses in some areas, hemorrhage, albumin precipitate in the glomerular spaces, fatty degeneration of the cortical ray tubules, and dilatation of some of the convoluted tubules; the intestine and stomach showed areas of necrosis of the villi and glands, respectively, with some submucous edema; the liver showed some central congestion, while in the lungs, hypostatic congestion, edema, and areas of subpleural hemorrhage were seen. Heart's blood urea, 329.1 mg.; urine (from pelvis) urea, 605 mg.; gastric contents' urea, 606.9 mg.; bile urea, 469.5 mg.

July 7-8, 1920.—Dog 13, yellow and white female, 6.5 kg., had both ureters ligated and cut. The animal became much depressed and died within eighty-four hours. Blood urea rose gradually from 26.9 mg. to 415.5 mg. Necropsy showed the usual fatty changes and hemorrhages in the kidneys, some hemorrhage in the duodenum, congestion and edema of the lungs. Heart's blood urea, 502.8 mg.; bile urea, 538.5 mg.; gastric contents' urea, 626 mg.; urine (in pelvis) urea, 780.8 mg.

SUMMARY

1. A series of three experiments was carried out on two dogs, demonstrating the rapid diffusion of urea into the tissues. Hence, within only a few minutes after the injection of 20, 30, 40, or 80 gm. urea, in divided doses of from 10 to 15 gm. at a time, the blood urea content was found to be very much what might be expected from the relationship between the weight of the blood in the body and the weight of the body as a whole. Thus, in a dog weighing 9.5 kg. the injection of 30 gm. urea gave a blood urea of 348.3 mg. per 100 c.c.; in a dog weighing 16.3 kg., 20 gm. urea gave 161.4 mg., 40 gm. urea gave 327.3 mg., and 80 gm. urea gave 491 mg. urea per 100 c.c. With the injection of from 30 to 40 gm. urea or more, salivation and vomiting were produced, but no other effects of any moment.

2. A second series of five experiments on five dogs was carried out to determine the toxicity of urea. The urea was injected practically continuously at the rate of 2 gm. urea (in 33⅓ per cent. solution) per minute. The average normal blood urea content of all the dogs used

was 20.7 mg. per 100 c.c.—the extremes being 11.9 and 29.5 mg., respectively.

In Dogs 2, 3, 6, 7 and 10, weighing 16.6, 9.0, 13.9, 16.0 and 8.9 kg., respectively, 202.3, 115, 105, 173 and 120 gm. urea, respectively, or, in other words, 1.2, 1.3, 0.75, 1.1 and 1.3 per cent., respectively, of the total body weight, an average of 1.1 per cent. were required to produce toxic symptoms such as generalized tremors and convulsions, coma and death. Hence, in order to produce serious symptoms, leading to death, one must inject, intravenously, at least 1 per cent. of the dog's weight in grams of urea. This confirms the results reported by Marshall and Davis.[45]

In this group of experiments there were rather broad variations in the symptoms produced, but certain symptoms were almost uniformly present. Among the earliest and most constant was salivation—of a thick, stringy, mucus type—appearing after from 25 to 50 gm. urea had been injected, and with distinct evidence of excretion of urea in the saliva. Thus, in Dog 6, after 50 gm. of urea had been injected, the thick saliva contained 412.8 mg. urea per 100 c.c.; in Dog 10, after injection of 56 gm. urea, the saliva contained 496.8 mg. urea per 100 c.c.

Vomiting was another constant symptom, usually coming on after 40 gm. urea, or more, had been given. It varied considerably among the different dogs, and was usually productive of gastric contents and some bile tinged mucus. It is interesting to note that in the only case (Dog 7) in which no convulsions were seen, vomiting was a very prominent symptom, and in this dog was seen the only example of chronic nephritis, affecting only one kidney but with very marked fibrosis.

In all the dogs of this series, after a sufficient amount of urea had been injected, increased reflex irritability, with nystagmus, tremors beginning in the head and neck and spreading downward, coarse twitchings, opisthotonic retraction of the head, tonic then clonic convulsions (except in Dog 7), unconsciousness, marked dyspnea, coma, diarrhea, and a gradual fall in temperature and pulse rate appeared in the order given, and lasted from a few minutes to many hours, most of the animals dying within twelve hours or less after the injection, two (Dogs 3 and 6) dying suddenly from respiratory paralysis. Bloody urination was rather a constant feature, the urine containing from 2,670 to 5,090 mg. urea per 100 c.c. While the picture presented by the animals varied somewhat in the relative predominance of its various elements, it could not be mistaken for anything but urea intoxication. If, after hours of twitchings and irregular panting respiration, with deep stupor and profound prostration, the animal recovered consciousness, it was very much depressed, weak, almost unable to stand, and

would lie in the cage totally unresponsive to ordinary stimuli, as if in a deep sleep, yet conscious.

The blood urea, determined about thirty minutes before or after the end of the injection, varied, of course, ranging from 669.5 mg. (rather low) in Dog 6 to 1,812 mg. in Dog 2, an average of 1,327 mg. urea per 100 c.c.

The necropsy findings in this series were strikingly uniform, consisting chiefly of marked hemorrhages in the submucosa and mucosa of the stomach and small intestine, with necrosis of the mucosa in some areas, subpleural hemorrhages with varying amounts of hypostatic congestion and edema of the lungs, and very marked fatty degeneration of the limbs of Henle's loop, with cloudy swelling of the convoluted tubules of the kidney. Apparently, therefore, the urea acted as an endothelial cell poison, and also as a toxic agent for renal epithelium.

The heart's blood urea content, postmortem, varied from 669.5 mg. to 1,702 mg. per 100 c.c., an average of 1,383 mg. The bile urea content varied from 1,242 mg. to 1,782 mg., an average of 1,514 mg. urea per 100 c.c., indicating an excretion of the urea in the bile, as might be expected from its ready diffusibility.

3. The third series of experiments consisted of the determination of the lethal dose of repurified urea (by recrystallization from alcohol), in order to exclude the poisonous action of any foreign substance, such as ammonium salts. In Dogs 8 and 9, weighing 11.5 and 10.7 kg., respectively, 130 and 118 gm. urea, respectively, were required to produce toxic symptoms, hence, 1.1 and 1.1 per cent. of the body weight, just as with ordinary commercially pure urea. The symptoms in this series, as well as the blood, bile and urine findings, and the necropsy observations, were entirely similar to those presented in the preceding series, and need not be discussed further.

4. In the fourth series, a single ureter was ligated doubly, or ligated doubly and then sectioned between ligatures, in four dogs, the animals being allowed to recover largely from the operation and then their tolerance to urea injection estimated.

In Dogs 5, 4, 14 and 15, weighing 12.5, 10.5, 10.3, 13.2 and 6.7 kg., respectively, 87.0, 63, 105 and 75 gm. urea were required to produce toxic symptoms, similar in every respect to those discussed above. Dog 4, curiously enough, received two separate injections, went into convulsions in each case, and recovered rapidly after both injections, although her symptoms were very much the same as those of the other dogs. In fact, this was the only instance of a dog recovering after convulsions. This animal was later operated on again and the remaining ureter ligated—as will be described under the next series.

It is seen, therefore, that in this series, with only one kidney functioning, the amount of urea necessary to produce toxic symptoms varied

from 0.6 to 1.1 per cent. of the body weight, an average of 0.8 per cent., as compared with 1.1 per cent .in animals with both kidneys functioning.

The general symptoms produced in this series were similar, in every respect, to those described in Series 2, and death occurred within about the same period of time after the injection. The blood urea content, determined shortly before or after the end of the injection, varied from 933 to 1,275 mg., an average of 1,078 mg. per 100 c.c. The bile and gastric contents urea showed distinct evidences of urea being excreted in those two fluids, particularly in the latter, in which the urea content averaged 1,815 mg. per 100 c.c. in two dogs. The urine urea, after the injection, varied from 1,690 to 2,040 mg. urea per 100 c.c.—distinctly low when compared with Series 2 and 3.

The necropsy findings in this series were, on the whole, quite similar to those in Series 2 and 3, except that the kidney corresponding to the ligated or divided ureter showed some hydronephrosis or pyonephrosis, of a moderate degree, with necrosis of, and hemorrhages into, the kidney tissue.

5. In the fifth and last series, four dogs were studied as to the effects of bilateral ligation and section of the ureters. These animals, Dogs 4, 11, 12 and 13, lived from three to four days, except Dog 11, which lived less than thirty-six hours. Unfortunately, not one of these animals was seen in the act of dying, so that while convulsions were never seen to occur, they may have taken place shortly before death. However, the absence of an early rigor mortis speaks against convulsions. In general, the symptoms were chiefly those of anorexia, a gradually falling temperature, an increasing drowsiness, and a marked depression, which was very obvious and constant. Irregularities in respiration appeared, but were not very constant. The blood urea rose steadily, reaching from 243 to 502.8 mg. per 100 c.c., an average of 383.7 mg. per 100 c.c. The bile urea varied from 273.0 to 538.5 mg. per 100 c.c., an average of 412.6 mg. per 100 c.c.; the gastric contents urea ranged from 350.0 to 626.0 mg., an average of 507.8 mg. per 100 c.c., indicating a distinct excretion of urea.

The necropsy findings in this series, of course, were those of degenerative changes in the kidneys, with a mild hydronephrosis, except in Dog 4, in whose right kidney (the right ureter was ligated long before the left) a very extreme hydronephrosis was present; also a suppurative peritonitis in the same animal. Dog 11 had a bronchopneumonic patch in one lung. The animals showed some hypostatic congestion and edema of the lungs, with a few subpleural hemorrhages. The stomach and intestines, in this series, were relatively unaffected, due, perhaps, to the low urea content of the blood as compared with that in the animals quoted in the second, third and fourth series.

CONCLUSIONS

The injection of urea, intravenously, in dogs produces a train of symptoms entirely analogous to that found in the convulsive or true uremia in man. Lesions are produced in the alimentary mucosa that may be related to uremic colitis. There seems to be a rather definite correlation between the severity of the symptoms and the concentration of urea in the blood.

Chronic uremia in man, ending ultimately in convulsions and coma, may be accounted for by urea intoxication, if we assume that the time element in the clinical cases is as important as the high concentration of urea in the animals, when injected. Obviously, more work is necessary, with particular reference to the use of such an apparatus as the Woodyatt machine for long continued injections of relatively low concentrations of urea. It is not implied here that only one factor is at play in the clinical forms of uremia, but urea retention must play a much more important rôle than that of an inert, harmless, waste product.

The asthenic condition produced by the ligation of both ureters involves probably many more factors than urea retention alone, and cannot, from the data at hand, be explained on any definite basis, except that such sudden occlusion of the ducts of one of the most important excretory systems must be followed by severe general metabolic disturbances.

Evidence was obtained that there is an active excretion of urea by the stomach, bile and intestine when excessive amounts are present in the blood.

I wish to thank Dr. Hanke of the Otho S. A. Sprague Memorial Institute for his helpful advice on several occasions and for purification of the commercial urea. I am deeply indebted to Prof. H. Gideon Wells for the suggestion of this problem and for his constructive criticism and cooperation throughout the entire time of this research.

METABOLIC STUDIES ON A CASE OF DIABETES INSIPIDUS *

I. M. RABINOWITCH, M.D.
MONTREAL, CANADA

Diabetes insipidus has been defined as a "chronic affection characterized by the passage of large quantities of normal urine of low specific gravity." [1]

The condition has been recognized for a long time, and the literature on the subject is large. Little, however, is found which bears on its study by the newer laboratory methods. Pathologists have furnished little gross or microscopic evidence as to the nature of this affection, except in those cases of the so-called "symptomatic type" in which an organic lesion in the brain has been found as a possible cause.

Experimentally, the possibility of a central origin for the condition has been suggested. Thus it has long been known that puncture of the floor of the fourth ventricle may produce a polyuria. No definite center for the control of polyuria, however, has been found.

Disturbances in the ductless glands have been suggested as the cause of this disease. These suggestions are based on the results obtained after the injection of extracts of the various glands. The evidence obtained is conflicting. The injection of pituitary extract has been shown to produce a polyuria, and cases in which a polyuria existed have been shown to improve after the administration of pituitary extract.[2] Although many experiments associate the pituitary body with a polyuria, such a specific action of this gland has been questioned. It has been shown [3] that a polyuria may be produced by a superficial lesion of the base of the brain, and this polyuria is greater than could be obtained after the injection of urea, caffein or sodium chlorid. A region has been localized (optopeduncular space) which seems to play some part in the mechanism of the regulation of water retention, with or without a polydipsia.

In 1914, Fitz,[4] in a review of the subject and the report of a case, summarizes the theories held as to the nature of diabetes insipidus as

* From the Department of Metabolism of the Montreal General Hospital.

1. Osler: Principles and Practice of Medicine, Ed. 8, p. 439.

2. Christie, C. D., and Stewart, G. N.: Study of a Case of Diabetes Insipidus, with Special References to the Mechanism of Diuresis and the Action of Pituitary Extract on It, Arch. Int. Med. **20**:10 (July) 1917. Schnabel, T. G., and Gerhard, A. H.: A Case of Diabetes Insipidus, New York M. J. **111**:812, 1920.

3. Camus, J., and Roussy, G.: Experimental Researches on the Pituitary Body, Endocrinology **4**:507, 1920.

4. Fitz, R.: Diabetes Insipidus, Arch. Int. Med. **14**:706 (Nov.) 1914.

follows: (a) That it is due to the inability of the kidney to excrete a urine of normal concentration; (b) that it is due to hyperfunction of the pituitary gland with a resultant diuretic action; (c) that it is due to a primary polydipsia with a resultant polyuria.

In the case reported by Fitz, the kidney function was found to be normal, except that it gave characteristic findings of a "vascular hyposthenuria, with the vessels sensitive to chlorid stimulation."

More recently, emphasis has been placed on the tissues of the body as a factor in the production of polyuria. As a result of certain chemicophysical changes, osmotic pressure, etc., the tissues of certain organs are unable to retain their full quota of water, and polyuria results.

In another theory an endeavor has been made to correlate a central origin with a renal factor,[5] and on this basis diabetes insipidus is regarded as a disease produced by the insufficiency or lack of internal secretion of the pars intermedia of the hypophysis, a secretion which normally regulates diuresis, by acting on the renal cells, causing vasodilatation of the arteries of the kidney.

The interesting problems involved, the numerous clinical methods now available for their study and the uncommon occurrence of diabetes insipidus in this hospital (the present case being the only one recorded in the last 50,000 admissions to the wards) have stimulated the metabolic study of the following case.

REPORT OF CASE

A male (Hospital No. 666-21), aged 47, was admitted Feb. 17, 1921, to the medical wards of the Montreal General Hospital, service of Dr. H. A. Lafleur, who made the clinical diagnosis. The patient complained of a craving for water and frequency of micturition.

Personal History.—Canadian farmer, married; no previous illness, except a radical mastoid operation in July, 1920, with, apparently complete recovery. Denies venereal disease.

Family History.—Irrelevant; no suggestion of syphilis.

Present Illness.—Apparently well until January, 1921, when he first noticed frequency of micturition and increasing thirst. The history is indefinite as to which of these appeared first. He consulted his physician, who asked him to measure his urine for twenty-four hours, when he found he voided seven quarts. Except for the large quantity, it was normal.

Physical Examination.—General examination negative, except for an old depressed scar at the site of a mastoid operation. Lungs negative.

Circulatory System: Blood pressure: systolic, 155; diastolic, 95; otherwise negative.

Nervous System: All reflexes were normal; no evidence of intracranial pressure, except that the spinal fluid during lumbar puncture was under great pressure.

5. Maranon, G.: Abstr., Endocrinology **5**:86, 1921.

CLINICAL LABORATORY REPORTS

Urine.—Clear, pale, acid reaction, no sediment, sp. gr., 1.002; no albumin; no sugar. Microscopic examination negative.

Blood.—Red blood cells, 4,864,000; leukocytes, 9,000; hemoglobin, 100 per cent. (Sahli). Differential count normal. Wassermann negative.

Spinal Fluid.—Clear; under great pressure; cell count, 9 per c. mm. all globulin tests negative. Wasserman negative.

Roentgenogram of Skull.—Shows no abnormality of the pituitary fossa, except that the fossa is shallow and the clinoid processes are indistinct. Sphenoidal sinus clear.

METABOLISM STUDIES

Since certain observations made are applicable to the study of more than one of the various problems which suggested themselves, and repetition of these observations was not practicable, they are recorded with comment only, but all are correlated under the general discussion.

GENERAL BLOOD CHEMISTRY

Nonprotein nitrogen	28.0	mg. per 100 c.c.
Urea nitrogen	16.0	mg. per 100 c.c.
Uric acid	1.1	mg. per 100 c.c.
Creatinin	1.2	mg. per 100 c.c.
Cholesterol	0.126	mg. per 100 c.c.
Sugar	0.100	per cent.
Chlorids (as sodium chlorid)	0.627	per cent. (plasma)
Total protein	7.4	per cent.
Albumin	4.6	per cent.
Globulin	2.8	per cent.

The only abnormal finding here is the hyperchloremia.

ENDOCRINE STUDIES

The close association of the function of the various endocrine organs has repeatedly been demonstrated. Since certain of these, e. g., suprarenal, thyroid and pituitary, have been shown to effect diuresis, these were studied.

BASAL METABOLISM

Apparatus used: Benedict respiration apparatus.[6]

Method.—The examinations were always made when the patient was in the postabsorptive state, and only after a preliminary rest period,[7] when the pulse rate, blood pressure and respiration rate were determined. The average normal oxygen consumption per minute, per square meter of body surface was taken from the tables of calories given by Drs. Aub and DuBois[8] using an average value of 4.83 calories per liter of oxygen, and an average normal respiratory quotient of 0.82. Under normal average conditions, 1 c.c. oxygen consumed has a value for heat production of 0.29 calories for each square meter of body

6. Benedict, F. G.: A Portable Respiration Apparatus for Clinical Use, Boston M. & S. J. **178**:867, 1918.

7. Boothby, W. M., and Sandiford, I.: Technic of Basal Metabolic Rates Determinations, Philadelphia, W. B. Saunders Company, 1920.

8. Aub, J. C., and DuBois, E. F.: The Basal Metabolism of Old Men, Arch. Int. Med. **19**:823 (May) 1917.

surface. The body surface was calculated from the formula of DuBois and DuBois[9] A-W 0.425 × H 0.725 × 7.25. After the normal basal values of the patient were found, the effect was noted after the administration of epinephrin chlorid, a pituitary extract and thyroxin.

During the period of this observation the blood pressure and pulse rate were taken every five minutes for two hours, and the basal metabolic rate was determined for a ten minute period at the end of 10, 30, 60, 90 and 120 minutes, respectively.

TABLE 1.—EPINEPHRIN CURVE

	Pulse per Minute	Blood Pressure		Respirations per Minute	Metabolic Rate	
		Systolic	Diastolic		C.c. Oxygen per Min. per Sq. Meter Body Surface	Percentage Deviation from Basal
Basal	72	150	96	24	134.3	..
0.5 c.c. epinephrin, 1:1,000 solution						
10 minutes later	76	152	96	24	147.6	11
30 minutes later	72	150	96	24	144.9	9
60 minutes later	70	148	96	24	134.3	1
90 minutes later	61	150	96	24	135.6	2
120 minutes later	72	146	96	24	131.6	1

The thyroxin was given after the epinephrin and pituitary extract tests were completed.

The normal findings under basal conditions do not justify the conclusion that there is no disturbance of the endocrine system, as it has been shown[10] that the basal metabolism is the result of the sum total activities of the various glands. So long as one gland shows hyperfunction and another shows hypofunction to the same degree,

TABLE 2.—METABOLIC RATE

	Metabolic Rate		Blood Pressure				Pulse		Respiration	
			Systolic		Diastolic					
	Basal	High	Basal	High	Basal	High	Basal	High	Basal	High
0.5 c.c. epinephrin 1:1,000	1	11	150	154	96	96	72	74	24	24
1 c.c. pituitary extract	2	4	156	164	92	92	74	80	24	24
10 mg. thyroxin six days previously	2	18	148	160	92	86	70	88	24	28

the basal metabolism may be normal. The remarkable subnormal response to epinephrin in this case, as compared with the average findings reported in normal individuals,[11] suggests the possibility of

9. DuBois and DuBois, E. F.: A Formula to Estimate the Approximate Surface Area if Height and Weight Be Known, Arch. Int. Med. **17**:863 (June) 1916.

10. Brock, S. R., and Kay, W. E.: A Study of Unusual Endocrine Disturbances, Arch. Int. Med. **27**:1 (Jan.) 1921.

11. Sandiford, I.: The Effect of the Subcutaneous Injection of Adrenalin Chlorid on the Heat Production, Blood Pressure and Pulse Rate in Man, Am. J. Physiol. **51**:407, 1920.

hypofunction of the chromaffin system. That this may play a part in the polyuria is possible, since it has been suggested [12] that the suprarenals act, in part, as a regulator of urinary excretion. To further investigate whether a hypofunction of the chromaffin system existed, the Csepai conjunctival,[13] Goetsch subcutaneous epinephrin test, and Ascoli subepidermal epinephrin test [14] were studied.

Csepai Conjunctival Test: Normally three drops of 1:1000 epinephrin solution causes a slight or moderate blanching of the conjunctional sac persisting from ten to twenty minutes. In this case, the blanching was slight and persisted for six minutes. This test was regarded as negative.

The Goetsch test was negative (Table 3).

Ascoli Subepidermal Test: Normally, 0.5 c.c. of 1:1,000 epinephrin solution causes the appearance of a swelling which, after a few seconds, assumes a dark inklike color. The swelling becomes surrounded

TABLE 3.—RESPONSE TO GOETSCH EPINEPHRIN TEST
(5 c.c. epinephrin 1:1,000 solution)

		Blood Pressure	
	Pulse	Systolic	Diastolic
Before administration	72	152	88
2½ minutes later	72		
5 minutes later	74	150	90
7½ minutes later	72		
10 minutes later	72	150	90
12½ minutes later	68		
15 minutes later	72	148	86
17½ minutes later	68		
20 minutes later	68	150	88
22½ minutes later	66		
25 minutes later	72	152	88
27½ minutes later	70		
30 minutes later	68	150	90
60 minutes later	72		
90 minutes later	70	148	88

by a pale halo of irregular projections, surrounded by a red zone. Normally, this reaction may be found, according to Ascoli, with dilutions even of 1:200,000.

In the case studied, with a 1:1,000 solution the blue-black central spot was present; there was slight blanching, but no red halo, and, therefore, this test was only slightly positive.

Ascoli pointed out that in the great majority of normal individuals the pathologic reactions to epinephrin and pituitary extract are dissociated and opposed. Since diabetes insipidus has been associated

12. McLeod, J. J. R.: The Endocrine Organs, Physiology and Biochemistry in Modern Medicine, Ed. 3, C. V. Mosby, St. Louis, p. 776, 1920.

13. Howard, C. P.: Functional Diagnosis of Polyglandular Disease, etc., Am. J. M. Sc. **158**:834, 1919.

14. Ascoli, M., and Fagiuoli, A.: Pituitrin Test, Endocrinology **4**:33, 1920.

with pituitary disease, tests for hypofunction of this gland were studied, including the Ascoli pituitary extract test [14] and that of Brock and Kay.[10]

Ascoli Test: Using the usual strength of obstetric pituitary extract as sold in the ampules, a reaction similar to that of a solution of epinephrin 1:1,000 is supposed to occur. This, contrary to expectations, did not occur, except as a slight suggestion of redness.

TABLE 4.—Results of Brock and Kay's Test

	Pulse	Blood Pressure	
		Systolic	Diastolic
Before administration	74	156	92
1 c.c. of pituitary extract injected			
5 minutes later	84	154	90
10 minutes later	74	164	90
15 minutes later	72	156	92

Although there was a slight rise in the systolic pressure, it was not marked enough, taking the personal equation of reading into consideration, to regard it as definitely positive.

Much clinical and experimental data tend to suggest that the pituitary body is concerned with carbohydrate metabolism. The finding of an increased sugar tolerance in acromegaly and pituitary tumors have been repeatedly reported. Keeton and Becht have shown that a hyperglycemia, associated with pituitary stimulation, is absent after transection of the spinal cord. Howard [13] states that a diminished sugar tolerance in the presence of other symptoms, suggestive of disturbance of the pituitary body, justifies a diagnosis of increased activity of the pars intermedia.

TABLE 5.—Sugar Tolerance Test

	Blood Sugar, per Cent.	Urine Sugar
8 a. m., fasting	0.125	0
100 gm. glucose given		
9 a. m.	0.206	+
10 a. m.	0.168	+
11 a. m.	0.120	0
12 a. m., 100 gm. glucose given		
1 p. m.	0.196	+
2 p. m.	0.162	+
3 p. m.	0.126	0

Contradictory findings have recently been reported.[5] Partial removal of one or both lobes of the pituitary body, or its total removal, did not appreciably modify the tolerance for carbohydrate, nor was it associated with the appearance of an alimentary glycosuria, and the injection of pituitary extract from the posterior lobe, anterior lobe or of the whole gland of the hypophysis did not sensibly modify—in the animals operated on—the tolerance for carbohydrate. Clinically,

however, the association of diseases of this gland and disturbance of sugar metabolism is definite. For this reason, the carbohydrate metabolism was studied. The method employed was the determination of the blood sugar, after a fifteen hour fasting period. Then 100 gm. glucose in 250 c.c. water flavored with lemon juice was administered, and the degree of hyperglycemia and glycosuria was determined at one hour periods, until the original findings had returned. The method used in determining the blood sugar was that of Folin and Wu.[15]

The blood sugar during the fasting period is rather high, with the method employed. Following the first administration of glucose, there is definite evidence of diminished tolerance, as shown by the glucosuria and suggestive evidence of delayed assimilation. The original concentration of the blood sugar was reached within the normal period, but the curve has a tendency to be rounded—not peaked. After the

TABLE 6.—DATA ON URINE FOR TWELVE HOURS

Time	Intake	Output, C.c.	Specific Gravity
8 to 9 a. m.	450	100	1.004
9 to 10 a. m.	300	450	1.002
10 to 11 a. m.	310	250	1.003
11 a. m. to 12 m.	220	90	1.004
12 m. to 1 p. m.	150	145	1.006
1 to 2 p. m.	550	110	1.007
2 to 3 p. m.	150	100	1.006
3 to 4 p. m.	150	260	1.001
4 to 5 p. m.	320	270	1.001
5 to 6 p. m.	340	200	1.002
6 to 7 p. m.	300	300	1.002
7 to 8 p. m.	300	250	1.002
8 p. m. to 8 a. m.	0	1,000	1.001

second administration of glucose, there was also a diminished tolerance, and practically to the same degree. This makes the test more positive, since normally an increased tolerance is expected after repeated injections.[16]

RÉSUMÉ.—In the study of the basal metabolism of a case of diabetes insipidus a normal rate was found. There was no evidence of increased activity greater than would be expected with the dose of thyroxin administered. A diminished sugar tolerance was demonstrated, suggesting hyperfunction of the pituitary gland, and hypofunction of the suprarenals was suggested by subnormal response of the basal metabolic rate to epinephrin and negative Csepai, Goetsch and Ascoli tests. A balance, apparently, is struck between those two abnormal functions in keeping the basal metabolism unaltered.

15. Folin, O., and Wu Hsien: A System of Blood Analysis, Suppl. 1, J. Biol. Chem. **41**:367, 1920.

16. Hamman, L., and Hirschman, I. I.: Studies on Blood Sugar, Bull. Johns Hopkins Hosp. **30**:306, 1919.

DIETARY AND KIDNEY FUNCTION STUDIES

As a preliminary observation, the patient was allowed the regular full hospital diet and as much water as he cared to take. The intake of fluids was measured and the time at which they were consumed was noted. The urine was collected every hour, and the volume and specific gravity were recorded.

It will be noted that although, on the whole, there is some relation between intake and output of fluids, the lack of a definite relation occurred during the hourly periods, from 8 to 9 a. m. and fro 1 to 2 p. m,. and again during the night when no fluid was taken. The persistent low specific gravity is marked. The total nitrogen for the day was 5.621 gm. and for the night 7.302 gm., which is apparently the total average excretion on a full hospital diet. This figure has frequently been noted during preliminary observations in mild diabetics, in whom there was not an increased metabolism.

TABLE 7.—RESULTS OF MOSENTHAL RENAL TEST MEAL

	Urine		Sodium Chlorid		Nitrogen	
Hour	C.c.	Specific Gravity	Gm.	Per Cent. Concentration	Gm.	Per Cent. Concentration
8 to 10 a. m.	90	1.020	0.108	0.120	0.921	1.02
10 a. m. to 12 m.	160	1.016	0.160	0.160	1.008	1.00
12 m. to 2 p. m.	135	1.013	0.432	0.320	1.285	0.95
2 to 4 p. m.	150	1.014	0.060	0.040	1.152	0.76
4 to 6 p. m.	115	1.015	0.092	0.080	1.255	1.09
6 to 8 p. m.	95	1.019	0.152	0.160	0.718	0.75
8 p. m. to 8 a. m.	390	1.020	0.780	0.200	5.400	1.04

Balance	Intake	Outout
Water	1,760	1,075
Salt	8.5	1.184
Nitrogen	12.6	11.799

The marked nocturnal polyuria and low concentration of nitrogen (0.456 per cent.) suggested impairment of kidney function, and seemed to support the theory that in diabetes insipidus, the kidneys are unable to excrete a concentrated urine. Therefore, the patient was placed on a Mosenthal renal test meal consisting of 12.6 gm. nitrogen, 8.5 gm. salt and 1,760 c.c. water, with the necessary restrictions. No food or fluids were allowed between meals. The meals were given at 8 a. m., 12 noon and 5 p. m., and the urine was collected at two hour periods from 8 a. m. to 8 p. m. and one specimen from 8 p. m. to 8 a. m. the following day. The possibility of the previous habits and diet influencing the test was excluded by giving the Mosenthal renal test for two days previous to the actual test.

The balance shows a slight water retention and more marked chlorid retention, and a normal nitrogen balance (allowing 10 per cent. excretion by the feces). The night urine is normal in amount and in concentration of nitrogen, but low in salt. The two hour specimens

show a normal concentration of nitrogen, but are low in salt. With the exception of the salt metabolism, the findings are normal. A striking feature is the ability of the kidney to excrete a urine of higher specific gravity on a restricted fluid diet than on an unrestricted one, and yet in the preliminary observations, there seemed to be no definite relation between the intake and output of fluids.

Since the renal test meal seemed to indicate that the metabolism of salt and water was at fault, observations were made with special reference to these. The problem was whether the kidneys were unable to concentrate salt or whether there were certain physicochemical changes in the tissues which removed the salt from the blood, and the salt did not reach a high enough concentration (threshold) to be excreted. That is a study was made of the relation between the concentration of the chlorid in the blood and its rate of excretion as described by McLean.[17]

The plasma chlorides were determined by the method of Whitehorn.[18] The normal threshold was accepted as 5.62 gm. per liter.

$$\text{Calculated plasma NaCl} = 5.62\sqrt{\frac{D \vee c}{W \times 4.23}}$$

$$\text{Calculated threshold} = \text{actual plasma NaCl} - \sqrt{\frac{D \vee c}{W \times 4.23}}$$

Where D — gm. per 24 hours.
Where C — gm. per liter.
Where W — weight in kilograms.

The collection of specimens was followed as described by McLean.[19]

	Plasma NaCl (gm. per liter)		
	Actual	Calculated	Threshold
Determined under ordinary condition.....	6.281	5.641	6.260
10 gm. NaCl	6.290	5.683	6.227
20 gm. NaCl	6.273	6.002	5.891
10 gm. NaCl + 10 gm. urea.....	6.286	6.001	5.905
10 gm. NaCl + 4 grains caffein-sodium benzoate	6.286	5.679	6.227
10 gm. NaCl + theobromin-sodium salicylate	6.261	5.682	6.201

RÉSUMÉ.—Judging from the normal values obtained by this method, there was a persistent hyperchloremia and a persistent high threshold. The administration of sodium chlorid stimulated the kidney to activity by lowering the threshold, and this effect was greater with the increased dose of sodium chlorid. The administration of urea, together with the small dose of sodium chlorid, did have some effect in lowering the

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threshold, but not to the same degree as the administration of more salt. The diuretics, caffein and theobromin, had no appreciable effect. The ability of the kidney to excrete nitrogen readily, as shown by the renal test meal, was corroborated by testing the effect on the blood and urine of the administration of urea.

The renal meal was repeated, but 10 gm. urea were given by mouth at breakfast.

TABLE 8.—Result of Second Renal Test Meal

	Urine		Sodium Chlorid		Nitrogen	
Hour	C.c.	Specific Gravity	Gm.	Per Cent. Concentration	Gm.	Per Cent. Concentration
8 to 10 a. m.	190	1.014	0.760	0.400	2.390	1.25
10 a. m. to 12 m.	215	1.012	0.860	0.400	1.806	0.84
12 m. to 2 p. m.	165	1.014	0.528	0.320	1.755	1.06
2 to 4 p. m.	160	1.015	0.896	0.540	1.747	1.09
4 to 6 p. m.	100	1.017	0.320	0.320	1.232	1.23
6 to 8 p. m.	90	1.019	0.216	0.240	1.285	1.42
8 p. m. to 5 a. m.	320	1.021	0.512	0.160	5.286	1.65
	1,240		4.092		15.501	

The balance shows that following the administration by mouth of urea there was a lessened tendency to retain salt and water and the nitrogen excretion was normal (allowing 10 per cent. excretion by stools and urea $= 2.14 \times$ Urea N).

The ability of the kidney to excrete nitrogen is well shown in Table 9. Although sufficient urea was given to raise the blood urea nitrogen to about 200 mg. per 100 c.c. (accepting 5 liters of blood as the average normal volume), the concentration of the blood urea nitrogen remained remarkedly constant.

TABLE 9.—Blood Urea

	Blood Urea Nitrogen. Mg. per 100 C.c.
8 a. m., before administration of 10 gm. urea (orally)	16.1
9 a. m.	18.2
10 a. m.	16.3
11 a. m.	17.4
12 m.	15.2

Résumé.—From these findings the conclusion is justified that there is an inability on the part of the kidney to excrete chlorids properly. That the kidney is unable to concentrate all solids is not proven since its ability to concentrate nitrogen is remarkedly good. The hyperchloremia, as found during the preliminary observations of this patient, is probably compensatory in nature, and favors excretion of sodium chlorid and is not a true retention. Clinically, there was no evidence of increase in weight, edema, etc. Since both a renal and hypophyseal factor are suggested in the case, observations were made on the effect of the administration of pituitary extract on the renal function.

The renal meal was then repeated, and 1 c.c. of a pituitary extract was administered hypodermically at the 8 a. m., 12 m. and 5 p. m. meals.

TABLE 9.—Result of Administration of Pituitary Extract

Hour	C.c.	Specific Gravity	Sodium Chlorid	
			Gm.	Per Cent.
8 to 10 a. m.	70	1.024	0.220	0.31
10 a. m. to 12 m.	130	1.017	0.410	0.31
12 m. to 2 p. m.	70	1.022	0.126	0.18
2 to 4 p. m.	150	1.020	0.510	0.34
4 to 6 p. m.	130	1.026	0.426	0.32
6 to 8 p. m.	100	1.030	0.316	0.316
8 p. m. to 8 a. m.	300	1.022	3.100	1.03
	950		5.108	

The injection of the pituitary extract was followed by a diminished diuresis, a retention of water and an increase in the salt excretion and concentration. That the concentration of salt is due to increased kidney activity and not only to retention of water is suggested by the greater excretion of salt and the analysis of the blood following the injection.

TABLE 10.—Sodium Chlorid in Blood

Actual, Gm. per Liter	Calculated, Gm. per Liter	Threshold, Gm. per Liter
6.002	5.689	5.933

It will be seen (Table 10) that the hyperchloremia has diminished, but in spite of this, excretion takes place at a lower threshold.

The effect of the pituitary extract soon wore off as shown in Table 11.

TABLE 11.—Intake and Output of Fluid Two Days Later

Time	Intake	Output, C.c.	Specific Gravity
8 a. m., breakfast			
8 to 9 a. m.	360	150	1.005
9 to 10 a. m.	200	400	1.001
10 to 11 a. m.	550	150	1.002
11 a. m. to 12 m.	320	360	1.001
12 m. to 1 p. m.	100	400	1.001
1 to 2 p. m.	260	265	1.003
2 to 3 p. m.	395	160	1.004
3 to 4 p. m.	410	175	1.003
4 to 5 p. m.	400	200	1.004
5 to 6 p. m.	150	260	1.005
6 to 7 p. m.	100	150	1.005
7 to 8 p. m.	280	200	1.006
8 p. m. to 8 a. m.	0	1,430	1.001

Résumé.—In the case of diabetes insipidus studied, no single specific lesion has been found to account for the polyuria.

Functional analysis of the endocrine system shows hyperfunction of the pituitary body and hypofunction of the suprarenals. A balance is struck between these two abnormal endocrine functions as shown

by the normal basal metabolism. Although the polyuria disappears on a restricted fluid intake, there is no evidence that the polyuria is due to a primary polydipsia. The kidney function is normal in every respect, except in its ability to concentrate salt. That the condition is due to physicochemical processes removing the salts from the blood and thereby not allowing a normal threshold to exist is not proven. The hyperchloremia found is apparently compensatory, aiding the excretion of salt and is not a true retention since there is no clinical evidence of salt retention. The administration of pituitary extract definitely caused a diminution of the polyuria and an increased rate of flow and an increased concentration of salt in the urine.

CONCLUSIONS

In a case of diabetes insipidus studied there is no one specific cause for the polyuria. An endocrine and a renal factor were found. Since the administration of pituitary extract improved not only the concentration but also the rate of excretion, it is suggested that the theory advanced that diabetes insipidus is produced by a lack of some internal secretion which normally regulates and moderates diuresis by acting on the renal cells holds in this case.